AROMA CHARACTERIZATION OF AMERICAN RYE WHISKEY BY CHEMICAL AND SENSORY ASSAYS

BY

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THESIS

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--Abstract--

American rye whiskey is a distilled, alcoholic beverage, manufactured and consumed in the United States since before the Revolutionary War. Although other whiskeys (Scotch, Irish, bourbon) have been studied extensively in recent years, the aroma profile and potent odorants of American rye whiskey have not been reported. Two dilution analysis methods for Gas Chromatography-Olfactometry (GCO) were used to identify the potent odorants in rye whiskey: Sample Dilution Analysis (SDA) – a novel, non-extractive, direct method of analyzing alcoholic distillates – and Aroma Extract Dilution Analysis (AEDA) – a widely used and well-understood extractive method. SDA was found to provide equivalent results to AEDA while reducing analysis time and avoiding extraction bias. American rye whiskey was found to be a complex aroma system, with no-one odorant responsible for its characteristic aroma, but among the key aroma compounds identified were: 3-methyl-1-butanol, 2-phenylethanol, *cis*-(3*S*,4*S*)-whiskey lactone, guaiacol, syringol, and vanillin. These compounds likely mainly originated from either yeast metabolism (in the case of fusel alcohols) or lignin pyrrolysis. Odorants identified as important through dilution analysis were then quantified using Stable Isotope Dilution Analysis (SIDA). All key odorants were quantified, with concentrations ranging from 2560 ppm (3-methyl-1-butanol) to 7 ppb (ethyl cinnamate). In addition, acetaldehyde was identified as a key odorant, and quantified using external standardization. Finally, model solutions based on the quantification were constructed and compared to authentic whiskey samples using a difference test: the R-Index by Rating method. It was shown that naïve judges were unable to discriminate between different brands of commercial whiskeys. These judges were also unable in some but not all cases to discriminate between the model and the commercial whiskeys, indicating that the model and the quantification it was based on were a partial success, but that further work is necessary.

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--1. Introduction--

American rye whiskey has seen a remarkable resurgence in the last 10-15 years. From near-extinction rye whiskey has been reborn, phoenix-like, with distillers both large and small scrambling to put rye on the shelf at all price points. Implicit in this resurrection is a conviction that rye whiskey has a unique aroma and flavor, different from bourbon, Scotch, Irish, or other whiskeys widely available. This perception is certainly widespread in lay literature (1) and is enshrined in United States law (2), but, as there is currently no published data characterizing rye whiskey's aroma profile, it is difficult or impossible to draw conclusions about its uniqueness.

There is, in fact, a remarkable lack of modern, detailed aroma analysis of American whiskeys in general. Poisson and Schieberle recently published an excellent analysis of the important odorants in a single brand of American bourbon whiskey (3, 4), but to this author's knowledge there currently exists no analysis of rye whiskey using modern techniques. It is therefore important to, first, subject a representative group of American rye whiskeys to aroma analysis, using modern techniques, in order to determine what makes rye whiskey a unique spirit. At the same time, one of the main barriers to this sort of research is its time-intensive nature. Each analysis requires rounds of painstaking sample preparation, instrumental analysis, model preparation, and, ideally, sensory studies. It is hypothesized that it is possible to streamline and optimize the process; this in turn will allow more complex and ambitious analyses, which will be more revealing and applicable to a broader range of situations.

Distilled alcoholic beverages like whiskey offer a unique opportunity to design an optimized process, since unlike most food products, they are able to be analyzed directly by gas chromatographic (GC) methods without extraction. Most food products are not suitable for injection into a capillary column system – they are full of lipids, proteins, and other non-volatiles that would irreversibly damage the equipment without revealing any important information about their composition. Alcoholic distillates, on the other hand, are usually 40-50% ethanol and 50-60% water, with the compounds of

interest, which distinguish one type of alcoholic distillate from another – volatile aroma compounds and non-volatile sugars, tannins, and other wood-extractives – making up less than 1% of the distillate by volume. Most foods must be extracted into solvents or sampled using headspace methods, but whiskey can be thought of already as an extract in an ethanolic matrix, and, with the proper GC setup, can be analyzed directly, without extraction or sampling.

This sort of non-extractive analysis has significant benefits. It helps to reduce the time required per analysis – as above, this sort of optimization is important for developing large scale analyses that can be used for industry or academic assays of alcoholic distillates. It also helps to avoid a fundamental problem with sample preparation: extraction (or sampling) bias. Each extraction is, ideally, representative of the food as a whole, but the solvent (or adsorbent in the case of headspace sampling) will extract (or adsorb) compounds with different polarities, moieties, or weights differently, depending on its own properties. Thus, a solvent may over-extract certain compounds, leading to an overestimation of their importance to the food, or an adsorbent may have no affinity for a certain compound, so, no matter how important it is to the food, it will not be detected in analysis.

The problem of extraction bias is usually dealt with by making multiple extracts with different solvents, in order to account for a wide range of possibilities, but this is time consuming, and avoiding an extraction step altogether is inarguably preferable. With alcoholic distillates, it is possible to inject the food product directly into a GC, the effluent from which can either be analyzed using olfactometric techniques (GCO) or using a mass spectrometer (GC-MS). In the present research rye whiskey was used as a subject to test the possibility of optimizing alcoholic distillate aroma characterization through the use of a non-extractive, direct-injection technique.

First, the important odorants in rye whiskey were identified through the use of dilution analyses, specifically Aroma Extract Dilution Analysis (AEDA), which was compared to Sample Dilution Analysis (SDA). AEDA, as the name implies, is an extractive technique which relies on correlating the number of

dilutions an extract must undergo before an odorant's impression is no longer obtained using GCO with the importance of that odorant to the food product as a whole; SDA is a non-extractive analogue of AEDA, relying on direct injection and GCO analysis of unextracted rye whiskey. AEDA is widely used and well respected, and so was used as something of a "gold standard" against which to judge SDA.

Once the important odorants in rye whiskey were identified using dilution analysis, the next step was to quantify these odorants in order to determine their concentration in whiskey. This was done using Stable Isotope Dilution Analysis (SIDA). In SIDA, stable-isotope analogues of each compound of interest are introduced into the food product; the food product is then usually extracted, and the extract is analyzed by GC-MS. Because the isotopic standards are physically identical to their unlabeled standards, but have unique mass spectra, it is possible to construct standard curves which, given a known concentration of isotopic standard, provide an accurate measure of the amount of unlabeled compound in the food. In the present research, rather than spiking a food and then extracting it, samples of whiskey were directly spiked with isotopic standards and then analyzed using direct injection into a GC-MS.

The experimentally determined concentrations of important odorants can then be empirically tested using several techniques. The first of which is an instrumental method, in which a model solution of the odorants at their calculated concentrations with an additional internal standard is analyzed by GC and compared to the actual whiskey spiked with the same internal standard at the same concentration. By first comparing the known internal standard, it is possible to compare peak areas directly between the two solutions, and to determine if the experimental concentrations are the same as those in the original whiskey.

Secondly, it is possible to compare the model solution to the whiskey using sensory methods. Poisson and Schieberle used Descriptive Analysis (DA) to compare their bourbon solution against their original whiskey (4) with positive results. However, DA does not actually give statistically verifiable

information on the confusability of different products. Therefore, the present research used a difference test – the R-Index by Rating Test – which is meant specifically to determine whether there is a significant difference between products. It is possible to judge with some sensitivity whether the model solution is confusable with the original product; confusability indicates that all significant compounds were identified and properly quantified.

American rye whiskey is a significant food product – economically, historically, and culturally. Yet, it has never been subjected to rigorous scientific study. The opportunity to do so is also an opportunity to develop and optimize a streamlined process for studying this and other alcoholic distillates. The present research, therefore, focuses at the same time on this particular beverage and on developing techniques to study a range of beverages.

--2. Literature Review--

I. INTRODUCTION

Since whiskey has been an economically important product throughout its history, it is not surprising that it has long been an object of scientific study. On the other hand, since whiskey is a privately produced product which, especially in the United States, is regarded as morally suspect due to cultural conventions surrounding alcohol consumption, published studies of whiskey are more scarce than one might imagine. Nevertheless, from the early 20th century, food scientists have used the tools at their disposal to study whiskey.

Whiskey is primarily composed of water and ethanol; the congeners – a term for any compounds in whiskey which are *not* ethanol or water – make up far less than 1% of the volumetric basis of a whiskey. However, it has been long known that this small fraction is what gives whiskey its unique character. Thus, much of the history of whiskey science is devoted to analyzing this congener fraction, including: how variations in production protocols affect it, what chemical compounds comprise it, and which of these compounds are actually important to the flavor of the finished whiskey.

The latter questions are those with which the current research is concerned, and so the previous research covered in this review will likewise be mostly concerned with historical and present attempts to analyze the congener fraction of whiskeys. Unfortunately, there is almost no published research on the particular characteristics of rye whiskey. Thus, this review concentrates on whiskey aroma research in general, and on research into American whiskey, like bourbon and rye, whenever possible.

II. HISTORY AND PRODUCTION OF WHISKEY

History of Whiskey Production

Whiskey is a generic term used to designate an alcoholic distillate from a cereal grain, usually barley, corn, rye, or wheat, in order of prominence of use. Whiskey is produced, consumed, and highly valued in many countries throughout the world, with Scotland, Ireland, The United States, Canada, and

Japan being the principal whiskey-producing nations. Currently, whiskey is one of the best-selling spirits in the world, with Scotch, American, and Irish whiskies accounting for approximately 17%, 12%, and 6% of the world spirits market, respectively (5). It is also one of the world's oldest distilled beverages. The term whiskey (sometimes spelled "whisky") originates from the Gaelic *uisge beatha*, meaning "water of life", a term with a meaning in common with the French *eau de vie*, Latin *aqua vitae*, and Scandinavian *aquavit* (6). Whiskey is thought to have originated in Irish monasteries in the 12th century, although it rose to its current prominence after being imported to Scotland in the 14th and 15th centuries (7).

Whiskey passed into the lay sphere after Reformation and became commercially important relatively quickly. The first recorded commercial transaction involving whiskey dates to 1494 (6). American whiskey developed during colonial times, as Irish and Scottish immigrants brought the knowledge of distilling to the United States. At first the settlers made whiskey primarily from rye, which grew well in the areas that would become Pennsylvania and Maryland. After independence from England, taxes levied by the new government on distilled beverages resulted in the "Whiskey Rebellion" of 1791-1794. As part of the resolution, the rebellious settlers resettled in Kentucky, in particular in Bourbon County. Corn (maize) was particularly suited to this area, and so became the principle cereal used in the whiskey made in that area. In this way the two principle types of American whiskey – rye and bourbon – evolved, and were given legal definition by Congress in 1964 (Bathgate, 2003).

American whiskey is different from other whiskeys both because of its location of origin and because of some unique steps in processing. To be called American whiskey, the distillate must be produced, matured, and bottled within the United States. First, the mash from which American whiskey is distilled is either composed of a majority of corn (in the case of bourbon) or rye (in the case of rye whiskey). Second, American whiskey, like Scotch whiskey, is double-distilled, but, unlike Scotch, it is distilled first to a relatively high proof (around 60% alcohol v/v) in a column still and then re-distilled in a "doubler" or continuous pot still. Finally, American whiskey is invariably aged in new, charred, American

oak barrels, a processing step which is thought to account for much of whiskey's characteristic aroma and flavor (5, 6).

Because whiskey's history is, in many ways, a defining attribute for consumers, whiskey production is based strongly in tradition and resistant to change. Modern processing technology, therefore, is applied to whiskey production mainly to optimize alcohol production, reduce waste, and otherwise optimize the cost/profit ratio. Nevertheless, whiskey, and particularly American whiskey, is an important product for several reasons. It is an immensely profitable section of an already profitable industry. In recent years, super-premium whiskeys (which retail at $40 a bottle and up) have grown in popularity. With an increasingly sophisticated marketplace willing to pay top dollar for premium products, the value of tradition and quality to whiskey producers is only increasing.

Of the two primary types of American whiskey, American rye whiskey is the main subject of this research. Rye whiskey is undeniably historically important: it can be considered the original whiskey of the United States, produced by Scottish and Irish immigrants to the mid-Atlantic region during the colonial period (6). Rye is generally considered to have a distinct character from other whiskeys. It is frequently described in contrast to bourbon, with bourbon earning descriptors like "sweet", "smooth", and "vanilla", while rye is often described as "dry", "spicy", or "complex" (1).

In recent years, rye whiskey has undergone a peculiar renaissance (1). After nearly disappearing from production post-Prohibition, rye whiskey continued to be produced only by a few large distilleries. Many consumers began to confuse American rye whiskey, which must be produced with a mash composed of at least 51% rye grain (2), with Canadian whiskey, a whiskey which, while it certainly contains rye, has different production protocols, and usually contains less rye than required by United States law. In the last 20 years, the United States and the world have seen interest in super-premium, small-batch, and artisanal spirits surge, and rye has been caught up in that movement. Rye is quickly becoming a boutique favorite, with hefty price tags and a devoted following.

Whiskey Production

American whiskey production is a complex set of related processes meant to facilitate and optimize the extraction of carbohydrates from cereal products and to convert these carbohydrates to an alcoholic distillate with the optimum organoleptic properties. In general, the production processes of rye and bourbon whiskey are differentiated only by the cereal mash; thus, this review touches on the general production of American whiskeys. First, the cereal grains are milled or cooked to make their constituent starches accessible. Through the use of native enzymes in barley, these starches are converted into maltose, which yeast are able to ferment into ethanol. Once this fermentation is complete, the resulting alcoholic solution (a beer of about 5-6% alcohol v/v) is distilled to a much higher proof (usually 65-70% v/v) and stored in new, charred, American oak barrels. The final product is then aged for several years, a processing step which, although human intervention is minimal, is fundamental to the quality of the resulting whiskey.

Whiskey has very few raw materials or ingredients: cereal grains, water, and yeast. The cereals traditionally used for American whiskey are rye and corn, with wheat and barley usually added in small amounts. Malted grain, usually barley, is important to whiskey making because the malting process, which involves sprouting and then drying the grain, facilitates the production of starch-breakdown enzymes in the sprouted grains (5). Barley, in the form of malted barley or malt extract, is particularly important because it contains high proportions of active α- and β-amylase, enzymes which convert the stored starch in cereals into sugars that yeast is able to utilize for fermentation. In most American whiskey barley (which has less starch) is kept to the minimum necessary to achieve full starch conversion. It is possible but unusual to malt rye in a similar fashion. Of the two main American whiskeys, bourbon usually contains about 70% corn, with the rest of the cereal content being divided between malted barley and rye, while rye whiskey usually contains about 51% rye, 39% corn, and 10% malted barley (6). Water, while nominally tasteless and odorless, can affect whiskey production due to

pH level or dissolved minerals; for example, it is generally thought that the iron-free limestone in Tennessee and Kentucky is important for their dominance of the American whiskey market (6).

The major American whiskey producers appear committed to large batch cooking for whiskey, rather than continuous processing (6). Once a mash percentage is decided upon, the grain, excepting the malted barley, is loaded into batch pressure cookers with water and steam injected to raise the pressure to 200 kPa for two hours, after which it is cooled to 62.5 °C and the barley is added to achieve starch conversion (8). This slurry, consisting of fermentable sugars, cereal fibers, and water, is then cooled fully for yeast inoculation and fermentation.

Generally, fermentation of the wort into beer is by a single, cultured strain of yeast, in order to avoid off-flavors and to maximize conversion of available sugar to ethanol. Distilling yeast is a subspecies of *Saccharomyces cerevisiae*, the species of yeast which is responsible for the fermentation of beers and wines, and is notable for being partially amylolytic, i.e., it is capable of fermenting starch-derived di- and tri-saccharides (9). While it is possible to hybridize or genetically engineer a yeast that is fully amylolytic, thus eliminating the need to rely on barley or rye malt for amylase, the law in some cases and distillers' adherence to tradition in all cases tends to prevent this. A typical fermentation runs 40-48 hours; although some evidence shows that short fermentations negatively impact the final spirit quality, longer fermentations increase the chance of microbial contamination of the wort, leading to off-flavor or reduced alcohol yield (5).

Besides aging in wood, distilling is probably the most important processing step for whiskey production. American whiskey is distilled twice, first in a Coffey, patent, or continuous still, and then in a simple pot still, known as a "doubler" or a "thumper" (figure 2.1). The continuous still is vastly more efficient than the pot still, so most of the concentration and separation of alcohol from water is effected in this distillation. The second distillation in the pot still further concentrates the alcoholic effluent

without removing as many congeners (non-ethanol volatile components, known to be responsible for flavor) as a second column distillation would, allowing for efficient production of a flavorful spirit (5).

Continuous, or Coffey, stills were developed for Scotch whiskey production in 1830 by Aeneas Coffey (5). They consist of two side-by-side columns (figure 2.2), called the analyzer and the rectifier. These can actually be thought of as a single column, divided in two for size considerations. The beer is preheated by passing in a closed circuit through the second column (the rectifier) and then fed into the top of the first column, the analyzer, while steam enters into the bottom of that column. As the beer falls down the analyzer, the steam causes volatiles to vaporize and pass with it into the rectifier, where the alcohol and water are separated. Each column consists, internally, of plates with holes large enough to allow vapor to pass upwards through them, and "downcomers", or tubes, connecting each plate, forcing the liquid beer (in the case of the analyzer) or condensate (in the case of the rectifier) to flow across each plate and receive maximum exposure to the up-flowing vapor (figure 2.3). Stills were traditionally made from copper, due to its heat tolerance and malleability, which, in a lucky coincidence, encourages inactivation and stripping of malodorous sulfur congeners (5, 6). Modern column stills still include copper for this reason, usually in the form of a pad of copper mesh at the top of the analyzer column (figure 2.1).

The doubler is a traditional pot still: that is, it is a large vessel, heated in most cases by steam (although direct firing is possible), capped with a "lyne arm", which encourages both reflux and the condensation of the alcoholic effluent (figure 2.4). The exact shape of the head of the pot still and the lyne arm is believed to deeply affect the flavor of the distillate, due to differences in volatile reflux and condensation (5, 7). In Scotch production, only pot stills are used, producing a highly flavored distillate, while in Canadian whiskey production only column stills are used, producing a very mild and inoffensive distillate. American whiskey production compromises by using a single column still followed by a pot still, in order to produce a whiskey that is at once highly-refined and flavorful. The distillate from the

column distillation, at around 125 °US Proof (62.5% alcohol v/v), is fed to the doubler, where it is distilled to around 135 °US Proof (67.5% alcohol v/v).

After distillation, the new distillate is aged in oak barrels. All whiskey, sold in the UNITED STATES, is aged in wood for some amount of time, but both bourbon and rye whiskeys, the major types of American whiskey, are required to be aged in new, charred oak barrels (2). It is now known that much of the characteristic flavor and aroma of American whiskey comes from the distillate's long contact with charred oak (10-12), the time of which, generally longer than 2 years, is written into laws for labeling for sale (2, 13).

The effects of aging and storage in barrels have been the subject of a great deal of research. Oak wood, which is the required wood for American whiskey aging, consists of approximately 45% cellulose, 15% hemicelluloses, 30% lignin, and 10% extractable volatiles, oils, sugars, and other organic substances (all % w/w) (5). Charring the inside of the barrels (figure 2.5) has a number of further effects: the creation of a layer of "activated" carbon capable of adsorbing "off-flavors" (14, 15), increased availability of oak extractives like "whiskey lactone", and the pyrrolysis of cellulose, hemicelluloses, and lignin, which produces a number of phenolic and heterocyclic aroma compounds with distinct smoky, sweet, and vanilla-like aromas, many of which are thought to be characteristic of American whiskey (10-12, 15) (figure 2.6). By law, American whiskey must be aged in new barrels, but a body of research shows that further re-use of barrels reduces both the activity of the activated carbon and the availability of the extractives, indicating that the new char is an important element of the production process (14).

Barrels of distillate are stored for differing lengths of time in warehouses. While most American whiskey is bottled between 2 and 10 years of age, whiskey has no theoretical upper limit on aging. The areas of the United States in which whiskey is primarily produced have climates that lead to a concentration of alcohol with aging (as water evaporates through the semipermeable barrels), in contrast to, for example, the Scottish climate, which generally leads to the evaporation of alcohol during

aging (the so-called “angel’s share”) (5). Thus, with no loss of valuable ethanol, American whiskey can be aged for extremely long periods. It is generally thought, however, that excessive aging in wood can lead to a loss of spirit character and domination by wood-derived flavor notes. It is possible to bottle at “barrel-strength”, which can be as high as 65-70% alcohol (v/v), but most American whiskey is sold diluted with pure water to 40-45% alcohol (v/v). Whiskey will continue to undergo slow chemical changes in the bottle, but if it is kept in a fairly controlled environment (without excessive light or temperature change) it should be stable indefinitely, since the high alcohol content acts as an extremely effective preservative and most aroma compounds in whiskey are not light sensitive, as they lack unsaturated carbon-carbon bonds (4).

III. AROMA COMPONENTS OF WHISKEY

Most important research into whiskey aroma has occurred after the advent of Gas Chomatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Olfactometry (GCO). Several published works before that period, however, offer interesting observations. Liebmann and Scherl (16) considered changes in an especially large sample of whiskey over a long period of time. The researchers aged 469 barrels of American whiskey – both bourbon and rye – in lots of five initially selected during 4 years, and monitored changes in major chemical constituents over the next 8 years. They concluded that the changes in whiskey during maturation were primarily due to extraction of barrel-wood constituents. Most interestingly, they found no difference in the components they measured between bourbon and rye whiskeys. Another study identified a number of phenolic compounds using steam distillation of whiskeys, but their importance to whiskey aroma was unable to be ascertained (17).

The advent of GC allowed for far more precise identification of minor constituents in whiskeys. By the early 1960s, a huge number of volatile compounds in whiskeys were being identified by a number of research groups (18-26). Unfortunately, most of these studies, while interesting from the standpoint of analytical chemistry and extraction and isolation science, mostly catalogued compounds, rather than

considering their importance. Many of them, for example, identified compounds hitherto unidentified in whiskeys (and sometimes in nature) (22), but were unable to determine whether these compounds made any important contribution to whiskey aroma.

Research which is more interesting by modern standards began to emerge in the 1970s and 1980s. Salo *et al* (27) were among the first to link sensory science and flavor chemistry in studying the aroma profile of whiskey. The same research group published research on specific compounds important to alcoholic beverages in general and whiskey in particular (28-31). In general, Scotch whisky (most commonly called "whisky" due to regional spelling variations) has been the whiskey of choice for study. Scotch whisky has the largest share of the whiskey market (5) and had a strong distillers' association willing to fund some research. Much of this recent research into whiskey has helped elucidate the impact of specific compounds on the flavor of whiskey.

It is well-known that the barrel used for maturing whiskey contributes much of the whiskey's flavor (15) – most, in the case of American whiskeys (5). Many of the important odorant classes in whiskey, especially lactonic and phenolic compounds, are extracted from wood during the maturation of the whiskey (32). Charring the interior of the casks, as is stipulated in the production protocols for American whiskeys, has a profound effect on the aroma profile of whiskeys aged in those casks, increasing positive sensory characteristics associated with mature whiskeys, such as *smooth*, *vanilla*, and *sweet*, while decreasing characteristics associated with immaturity, such as *pungent*, *sour*, *oily*, and *sulfury* (14). These changes were evident as soon as 3 months after the distillate was introduced to charred barrels. It has also been shown that these wood extractives affect the partitioning of esters and the solute interactions of water and ethanol (33). Thus, it is obvious that the charring of barrels in the production of American whiskey has a profound effect on the ultimate flavor profile.

One of the most important odorants extracted from wood in aged distilled beverages is a branched γ-lactone which is often referred to as "oak lactone" or "whiskey lactone", and is more

systematically called β-methyl-γ-octanolide. It appears that Suomalainen and Nykanen were the first to report the presence of oak lactone in aged distilled liquor (12). Otsuka *et al* identified the two stereoisomers that occur in aged liquors, namely *cis*-(3*S*, 4*S*) and *trans*-(3*S*, 4*R*) (26). As can be gathered from the common name "whiskey lactone", it has been considered an important component of whiskey aroma, conveying a pleasant, woody-sweet aroma at low concentrations and a sweet-coconut aroma at higher concentrations (12). However, while Wanikawa *et al* found correlation between positive attributes in descriptive analysis and lactone content, they did not specifically correlate this with oak lactone (34). Oak lactone is usually one of the most important, but not *the* most important, compounds in dilution analysis of whiskey (3, 4).

Lee *et al* (35) reviewed the origins of flavor in Scotch whisky, from production to flavor compounds to sensory research. The researchers designed a flavor wheel meant to comprehensively encompass all sensory percepts heretofore identified in Scotch, and compounds found in Scotch which gave rise to these flavors and aromas. This flavor wheel offers valuable insight into the possible composition-function relationship between flavor compounds in Scotch and its sensory impact. The same group showed that certain types of sensory perceptions are correlated with quality-levels of Scotch, with the "deluxe" Scotch category characterized, for example, by *fruity*, *buttery*, *malty*, and *nutty*, and "retailer" (cheap) Scotch as *solventy*, *soapy*, and *rancid* (36). Unfortunately, while Scotch and rye whiskey are related, they have significantly different sensory attributes, and so these whiskeys must have different chemical compositions.

Unlike Scotch, bourbon whiskey is substantially similar to rye whiskey; they differ only in their "mash bill" – the distribution of cereal grains in the mash fermented for distillation. Aroma research on bourbon using GCO/GC-MS has been fairly scant, but several studies stand out.

Poisson and Schieberle (3) used liquid-liquid extraction, fractionation, Solvent-Assisted Flavor Evaporation (SAFE), and dilution analysis (AEDA) to identify the most potent odorants in a single

American bourbon whiskey. They also identified highly volatile aroma-active compounds by static headspace olfactometry (SHO) and dilution analysis. Altogether, 45 compounds with dilution factors (FDs) over 5 were identified in the extract, while 23 aroma-active compounds were identified in the headspace. Of these compounds, β-damascenone, γ-nonalactone, *cis*-(3*S*, 4*S*)-β-methyl-γ-octanolide (whiskey lactone), γ-decalactone, eugenol, and vanillin were identified as the most potent odorants, being the compounds with the highest FDs (above 10 dilutions in dilution at a factor of 2). Because the compounds identified by these researchers are substantially similar to those expected and identified in rye whiskey, the chromatogram, FD-chromatogram, and table of important odorants found in the solvent extract is reproduced here (figures 2.8 and 2.9).

The same researchers quantified the compounds identified as important odorants in bourbon whiskey using stable-isotope dilution analysis (SIDA) (4). They then used the quantification data in order to construct model whiskeys and conduct aroma omission studies, which in turn allowed them to validate the importance of the compounds identified through AEDA. They successfully quantified 31 of the 45 important odorants previously identified (figure 2.10) using SIDA. They then constructed model whiskeys using the quantification data, and conducted sensory omission studies, confirming to their satisfaction that they had constructed accurate models, and therefore had identified the most important odorants in bourbon whiskey. It is worth noting, however, that they used only an orthonasal (sniffing) sensory test – a retronasal (tasting) test might provide more interesting results.

IV. RELEVANT TECHNIQUES IN INSTRUMENTAL AROMA RESEARCH

Extraction Methods

In general, the volatile compounds in a foodstuff are responsible for the characteristic aroma of that product (37-40), but it is important to note that not all volatile compounds contribute significantly – or, indeed, at all – to the aroma of a product. Most instrumental aroma analysis requires the isolation of

the volatile component. While there are multiple methods of volatile isolation, the most relevant to alcoholic beverage analysis are Solid-Phase Microextraction (SPME) and solvent extraction (39, 40).

SPME is solvent-free method of volatile isolation – a fiber, coated with adsorbent material, is exposed either to the headspace of a food product or, in some applications, is submerged directly into a liquid product (41). SPME offers several advantages, including reproducibility, high throughput, and a lack of environmentally-damaging or health-hazardous solvents. It has been applied with some success to analysis of whiskeys (41, 42). Unfortunately, SPME is also expensive, and competitive behavior of compounds towards adsorption and with different coatings can result in failure to obtain a representative volatile sample of the food product (40). In the case of solid foods, SPME can only be used to analyze headspace.

Solvent extraction relies on direct liquid-liquid extraction of a food product by an appropriate solvent. In alcoholic beverage analysis, the most common solvents used are diethyl ether, pentane, dichloromethane, and various Freons (chlorinated and fleuronated methanes) (39, 40). One of the main considerations in alcoholic beverage extraction is limitation of ethanol extraction; ethanol is a volatile, odor-active compound, but is usually of limited interest to researchers. The use of highly non-poplar solvents can help eliminate ethanol extraction. Solvent choice may also rely on the specific compounds on which the research focuses, or, in some cases, solvent choice may be the *raison d'etre* of the research itself: a study of a large range for solvents for producing a representative beer extract showed that a dichloromethane extract was most representative, while a study of champagne used an ethanol demixing technique to produce the most representative extract (40).

Unfortunately, this demonstrates the main weakness of solvent extraction: the extract obtained will vary depending on the solvent used. While techniques like SIDA can account for bias in compounds detected after extraction, it cannot account for compounds that may be completely excluded from extraction, or compounds so volatile that they are entirely lost during extraction.

Direct Analysis of Alcoholic Beverages by Gas Chromatography

The chromatographic analysis of the aroma of a food usually involves the extraction of the aroma fraction of that food into an appropriate solvent. Distilled alcoholic beverages, however, can in fact be thought of as aroma extracts in an ethanolic solution. Since this ethanolic solution is composed of ethanol, a volatile solvent, and water, it is theoretically possible, given the correct injection procedure, to directly inject this distilled flavor extract into a gas chromatograph for analysis.

There are two methods of sample injection commonly used in aroma research: split/splitless and cool, on-column injection (43). Split/splitless inection allows for the injection of a relatively large sample, which is not necessarily clean (that is, the sample may contain some nonvolatile material that ideally should not be introduced to the column). During injection excess solvent can be vented, while impurities in the sample remain in the inlet liner, and are not transferred to the column. Unfortunately, the high temperatures required for this injection can break down labile compounds and form artifacts. Furthermore, heavier (less volatile) compounds are not transferred to the column as effectively as lighter (more volatile) compounds, resulting in the introduction of an inlet bias to the analysis. On-column injection, which involves introducing a small volume of sample directly into the column or pre-column, eliminates any bias by transferring all of the sample directly to the column. Unfortunately, because the sample is transferred completely, only small volume injections are possible, and the sample must be exceedingly clean to avoid damage to the column from non-volatile contaminants. Thus, neither method is ideal for direct analysis of whiskey.

Programmable Temperature Vaporizer (PTV) inlets, a relatively recent innovation, offer a compromise between the sensitivity and accuracy of on-column injection and the robustness of split/splitness injection. PTV inlets can be set up to run in cold splitless mode, which allows for large volume injection with relatively good (85-90%) transfer and no artifact formation (44). Direct injection

of alcoholic beverages using PTV inlets for chromatographic analysis has been examined in several recent studies.

Da Porto *et al* (45) used direct injection *without* a PTV inlet, using hot split injection, to evaluate a novel orange spirit using GC-MS. Unfortunately, due to their lack of appropriate injection protocol, excessive ethanol transferred to the column and affected retention indices, making compound identification difficult. Madrera *et al* (46) used direct injection with more success, again without inlet temperature programming, to analyze both major and minor constituents of a cider distillate by GC-MS. Macnamara *et al* (47) showed that accurate identification and quantification using cold splitless direct injection of whiskey was possible. None of these researchers, however, applied direct injection with any GCO techniques, dilution or otherwise, and so these studies did not determine whether the compounds identified were the important aroma compounds in the samples.

Dilution Analysis in Aroma Research

Dilution analysis is a technique that was developed for use with GCO in order to identify potent odorants and Odor Activity Values (OAVs) without going through the laborious work of determining thresholds of all compounds involved. Two main types of dilution analysis are in use today: Charm Analysis, a technique pioneered by Dr. Terry Acree at Cornell University, and Aroma Extract Dilution Analysis (AEDA), a technique developed by Dr. Werner Grosch (48, 49). The use of dilution analysis with GCO has been detailed in a number of reviews (37, 38, 48-51), including in the study of alcoholic beverages (40). Rather than reiterate the work included therein, this review will focus on AEDA, the technique which is used and modified in the current research.

AEDA is used more widely used than Charm Analysis, but both techniques proceed by serial dilution: a sample is diluted serially and analyzed by GCO, with the GCO operator noting for how many dilutions each individual aroma peak persists. In AEDA, the number of dilutions in which a particular compound is detected is called its flavor dilution (FD) value or factor. The FD value of a compound is

directly proportional to its OAV. In general, the compounds identified with the highest FD values in AEDA are considered most likely to be important contributors to the aroma of a food (48-50).

Unfortunately, while it is a useful screening procedure, dilution analysis in general and AEDA in particular cannot be relied upon to directly identify the key odorants of a food product for a number of reasons. First, AEDA relies on an aroma extract – it is impossible to inject, for example, a piece of cheese directly into a gas chromatograph – and so one cannot correct for any losses of volatiles during extraction, i.e., extraction bias (48). Thus, highly volatile compounds or compounds that are poorly extracted by the chosen solvent will be under-represented in AEDA, while compounds that are well extracted will be over-represented.

Second, AEDA relies on the linearity of odor stimulus response to dilution, and that all compounds respond with the same sensitivity to dilution (that is, the slope of the assumed linear relationship is the same between all compounds) (38). Unfortunately, this assumption is not necessarily true. For example, β-damascenone, a norisoprenoid important to many foods, including most whiskey, has a very low threshold, but fails to increase in intensity appreciably over large concentration ranges – that is, it has a very low response to dilution, and possibly a non-linear relationship between odor stimulus and dilution.

Finally, AEDA relies, indeed, depends on the use of gas chromatography, meaning that the OAVs which are obtained through AEDA are OAVs in a neutral gas matrix, which is rarely equivalent to the food matrix from which the compounds have originally been isolated (38, 49, 49, 50). Additionally, using a separatory method like GCO means that potential synergistic or suppressive effects between compounds are eliminated (51).

Nevertheless, AEDA has been used successfully to reconstruct aroma models of alcoholic beverages (4). It is possible that alcoholic beverages, which have a relatively simple matrix and fairly stable volatile components, are exceptionally good candidates for this kind of dilution analysis. It is

further possible that direct injection of the alcoholic beverages, as is discussed in the following section, may help eliminate some of the extraction bias inherent in dilution analysis.

V. SENSORY ANALYSIS OF WHISKEY

The data obtained by instrumental analysis of food products by GCO is an important part of the complete picture of the chemical basis for the aroma profile of that product. However, it is important to remember that "GC presents odorants that are completely volatilized in air" (37). GC techniques provide investigators with an important opportunity to consider and analyze individual components in a controllable, context-less environment. Unfortunately, food products are complex mixtures of proteins, lipids, and carbohydrates which can dramatically affect the release, and therefore the actual impact upon consumption, of specific aroma compounds. Furthermore, there is a possibility of synergistic or antagonistic interactions between aroma compounds, or of abundant aroma compounds affecting the food environment itself (52, 53).

It is therefore clear that, if authoritative statements about the importance of certain aroma compounds in food are to be made, these compounds, which are usually identified by GCO and extractive methods, must be evaluated in the context of the original food matrix, or in a matrix as similar to the original food as possible. Most commonly, model studies are used, in which an odorant mixture based on important odorants identified and quantified through chromatographic methods is added to a deodorized matrix. This odorant model is then compared, using sensory science methodology, with the original food product. If the model is a satisfactory match for the original food product – that is, it is confusable with the original - omission studies can be employed, in which various models are constructed in order to see which compound(s), when omitted, causes the model to differ most profoundly from the original (49). These studies can provide insight into which compounds are most important, and confirm identifications from chromatographic methods like AEDA.

In most cases it is difficult to provide a deodorized matrix which is identical to that of the original food. However, in the case of alcoholic distillates like whiskey, an accurate matrix is much easier to construct. Apart from some dissolved, non-volatile components extracted from barrels during the aging process, the whiskey matrix is nearly a pure mixture of ethanol and water. Deodorized non-volatiles can be obtained by the use of Solvent Assisted Flavor Evaporation (SAFE) (54), a technique which gently distills volatile fractions away from non-volatiles using extremely low pressure and moderate temperature. While SAFE normally is used to gather volatiles, the remaining residue after distillation of whiskey is the totality of the non-volatile components in whiskey (4). Thus, it is relatively simple to construct a deodorized whiskey model for use in sensory studies.

A number of sensory techniques have been used to link data from instrumental analysis of whiskey composition and the sensory properties of whiskey. Piggott and Jardine (55) have summarized the use of descriptive sensory analysis in whisk(e)y research. From the same research group, Clyne *et al* (14) interpolated chemical data from GC-analysis with descriptive sensory analysis to determine the role of cask charring and wood type on the sensory profile of Scotch whisky. While they found significant differences in levels of phenolic and lignin-derived compounds between whiskies aged in charred and uncharred barrels, these differences did not completely account for the sensory differences determined by descriptive analysis. Piggott *et al* (56) used a similar interpolation of instrumental and descriptive sensory analyses to compare Scotch whiskies aged in casks with different histories (from port, Madeira, bourbon, etc. maturation). They concluded that sensory data indicating an increase in mature, desirable characteristics correlated well with maturation time, and somewhat with barrel type.

Lee *et al* reviewed the current knowledge of the chemical and structural origins of sensory characteristics in Scotch whiskies (35). In regards to its physical composition, they note that whisky is a hydroalcoholic microemulsion with partitioning of wood- and grain-derived aroma compounds significantly affected by wood-maturation (57). Researchers found that while consumers use a fairly

casual recognition judgment to determine whether a whisky is acceptable, industry members tasked with quality judgments use a more granular assessment of different sensory attributes to determine whisky quality and to make blending and other processing decisions. The researchers constructed a flavor wheel to represent groups of sensory characteristics which may be found in whiskies, and suggested chemical analogues for each characteristic. Unfortunately, many sensory attributes found in Scotch whisky, such as *peaty* (smoky, medicinal, burnt, meaty flavors) are not necessarily present in rye or bourbon whiskeys, just as some of the compounds responsible for these characteristics, like heterocyclic nitrogenous compounds, may not be present in relevant concentrations in American whiskey (58).

Sensory analysis, of course, has been applied not only to whiskeys, but to nearly all classes of alcoholic distillates. Peña y Lillo *et al* (59) compared a trained sensory panel's ability to discriminate between samples of pisco (an un- or lightly-aged grape spirit produced in several South American countries, in this case Chile) using orthonasal (sniffing) or retronasal (tasting) methods. They found that the panelists discriminated the samples slightly better using orthnosasal methods, although there was a large degree of variation between panelists and between spirit samples. The variance between samples, and the non-linear behavior of the "oak" attribute may indicate that tasting may provide better discrimination in some cases, such as when a large portion of the aroma compounds in a sample are derived from aging in wood.

Aside from problems with the potential for intoxicated panelists, the use of taste-directed sensory analysis of alcoholic distillates is a tempting prospect. Highly alcoholic distillates like whiskey have the potential for being difficult to analyze by nose, even for experienced assayers. In a review and refinement of guidelines for sample handling in the Scotch whisky distilling industry, the author noted that samples should always be provided at 20% abv (%v/v), in order to avoid olfactory fatigue for the assessors (60). Not only can the high ethanol content of whiskey cause fatigue, but varying

concentrations of ethanol can affect odor perception and release, both physicochemically and perceptually, through synergistic and antagonistic sensory interactions (61).

It is thus apparent that sensory analysis of whiskey is an important adjunct to chemical-analytical methods for empirically determining important odorants. Without sensory analysis, it is not necessarily clear that compounds identified as potentially important are significant in actuality. At the same time, sensory analysis of a highly ethanolic distillate like whiskey presents certain challenges. In sensory analyses of whiskey, it is necessary to present samples to assessors in a way that allows them to "see past" the strong effects of ethanol, and assess the actual distinguishing characteristics of each whiskey. It is possible that a combination of orthnoasal and retronasal samplings will provide one solution to this problem.

VI. FIGURES AND TABLES

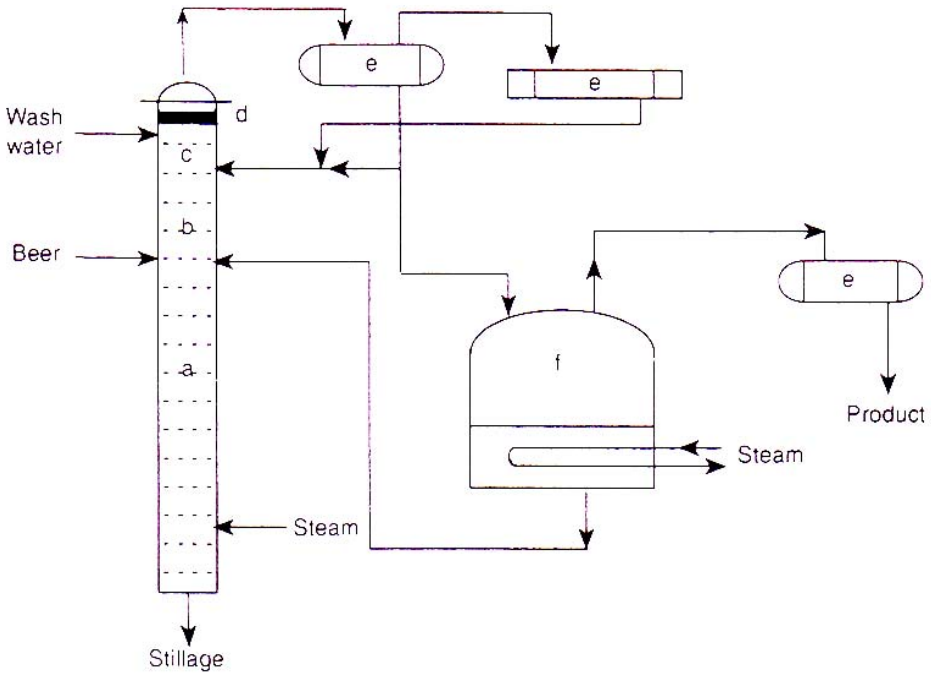


Figure 2.1: Schematic flow diagram of bourbon beer still and doubler: a) beer still stripping section; b) entrapment plate; c) rectifying section; d) copper demister; e) condensers; f) doubler (5), originally from Watson (62).

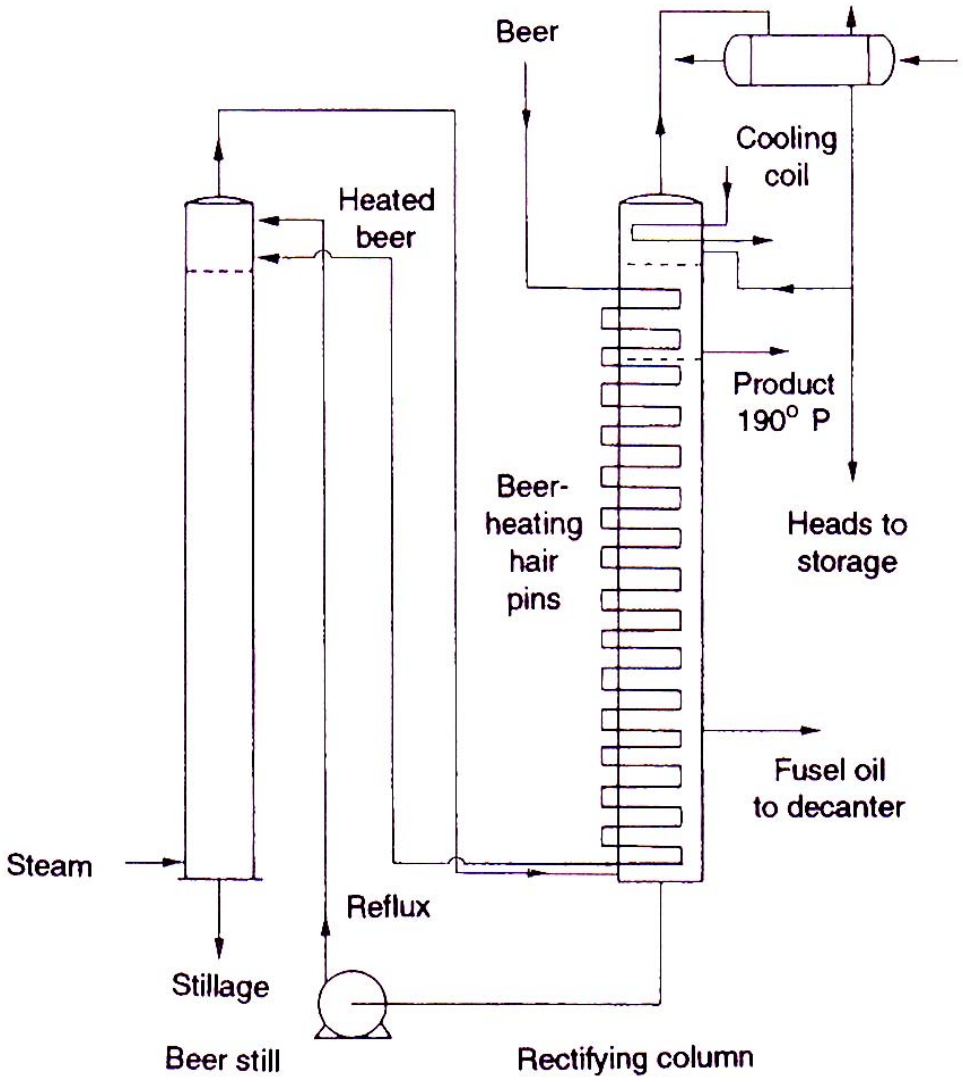


Figure 2.2: Schematic for a continuous or "Coffey" still (5), originally from Panek and Boucher (63).

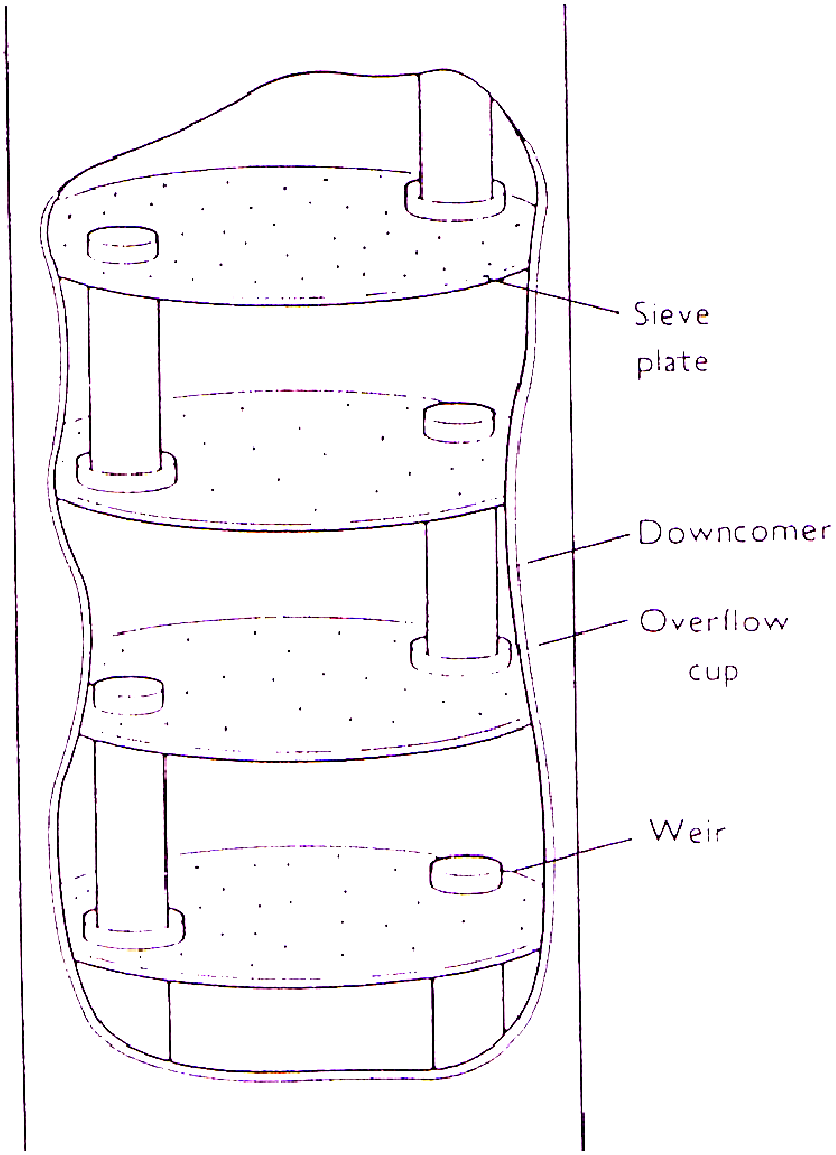
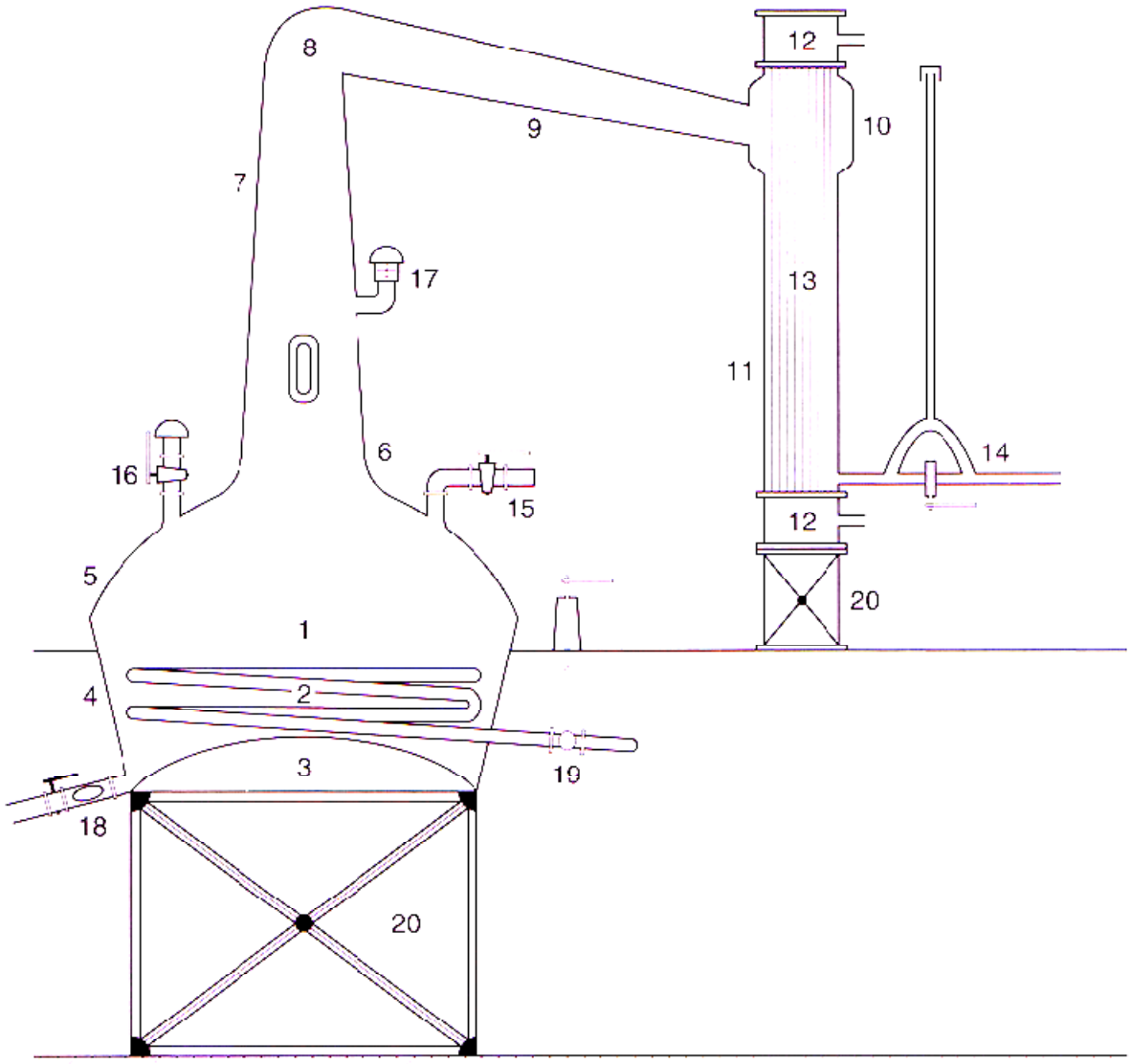


Figure 2.3: The construction of a typical distillation column, illustrating construction meant to facilitate cross-flow (5), originally from Panek and Boucher (63).



Key

1. Pot
2. Steam heating coil
3. Crown
4. Flue plate
5. Shoulder
6. Ogee
7. Swan neck
8. Head
9. Lyne arm/lye pipe
10. Vapour chamber
11. Shell and tube condenser
12. Water jacket
13. Tube bundle
14. Tail pipe with siphon
15. Charging line/valve
16. Air valve
17. Anti-collapse valve
18. Discharge line/valve/sight glass
19. Steam line/valve
20. Cradle

Figure 2.4: Pot still diagram (7).

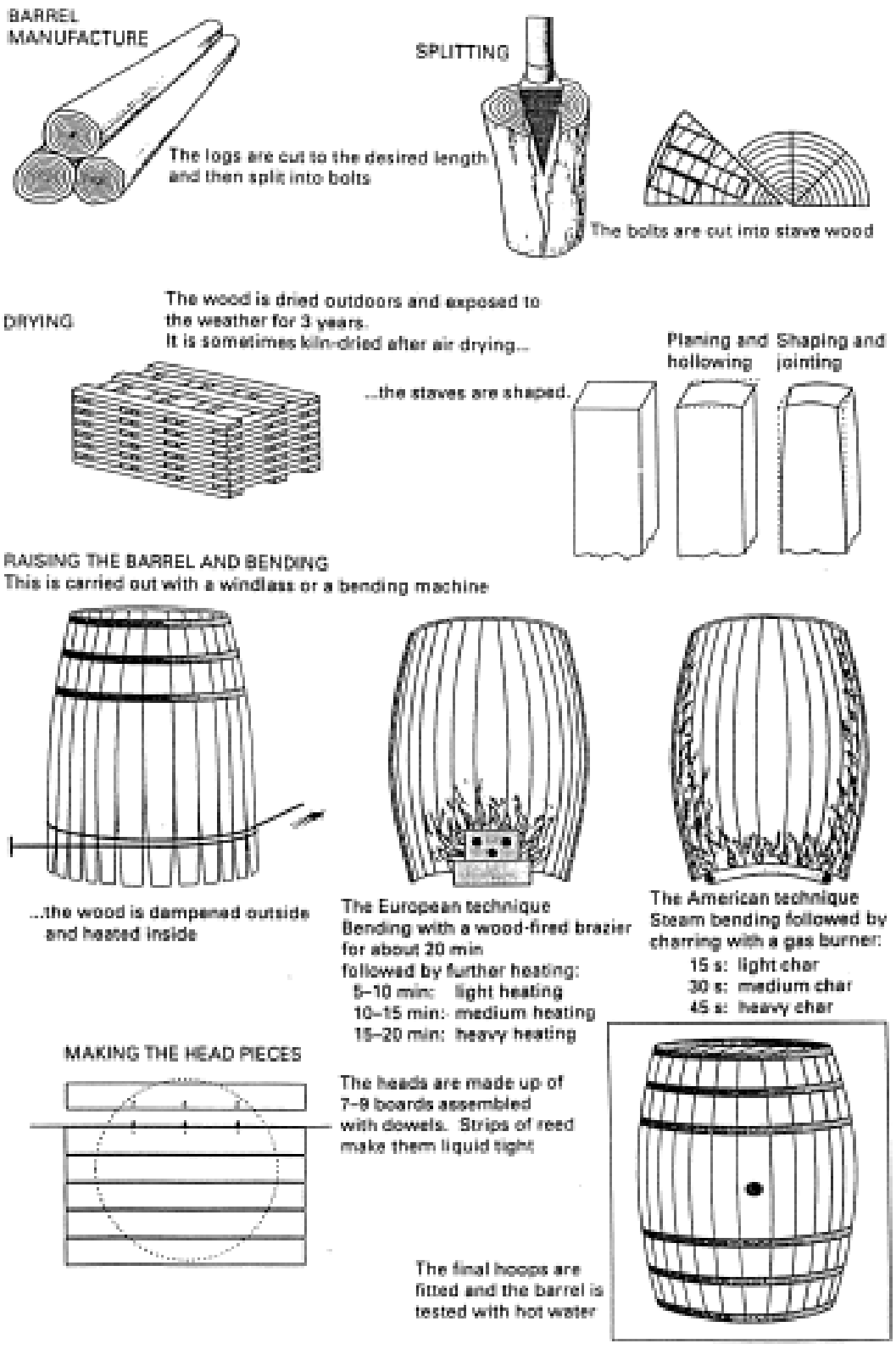


Figure 2.5: Barrel production for wood-aged ethanolic distillates (15).

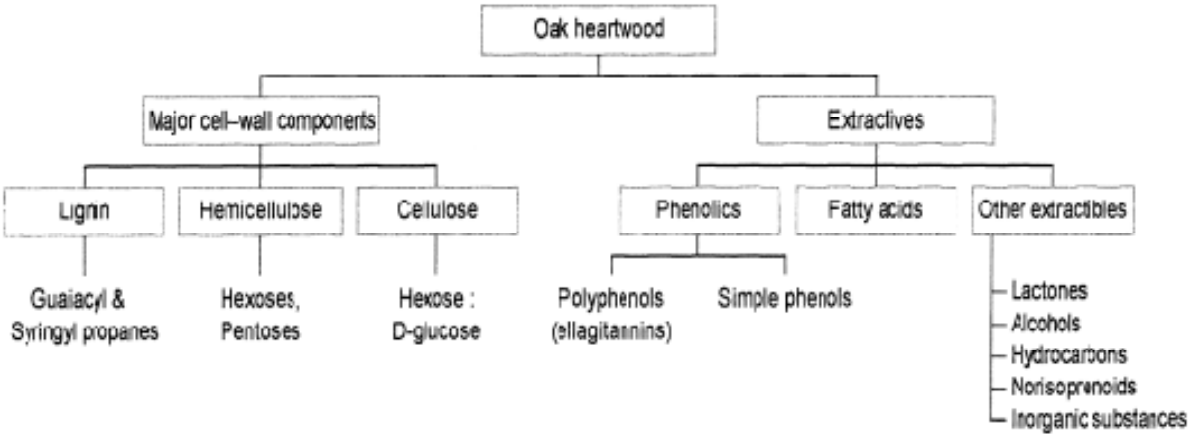


Figure 2.6: Ethanol-extractable breakdown products of oak used in cooperage (15).

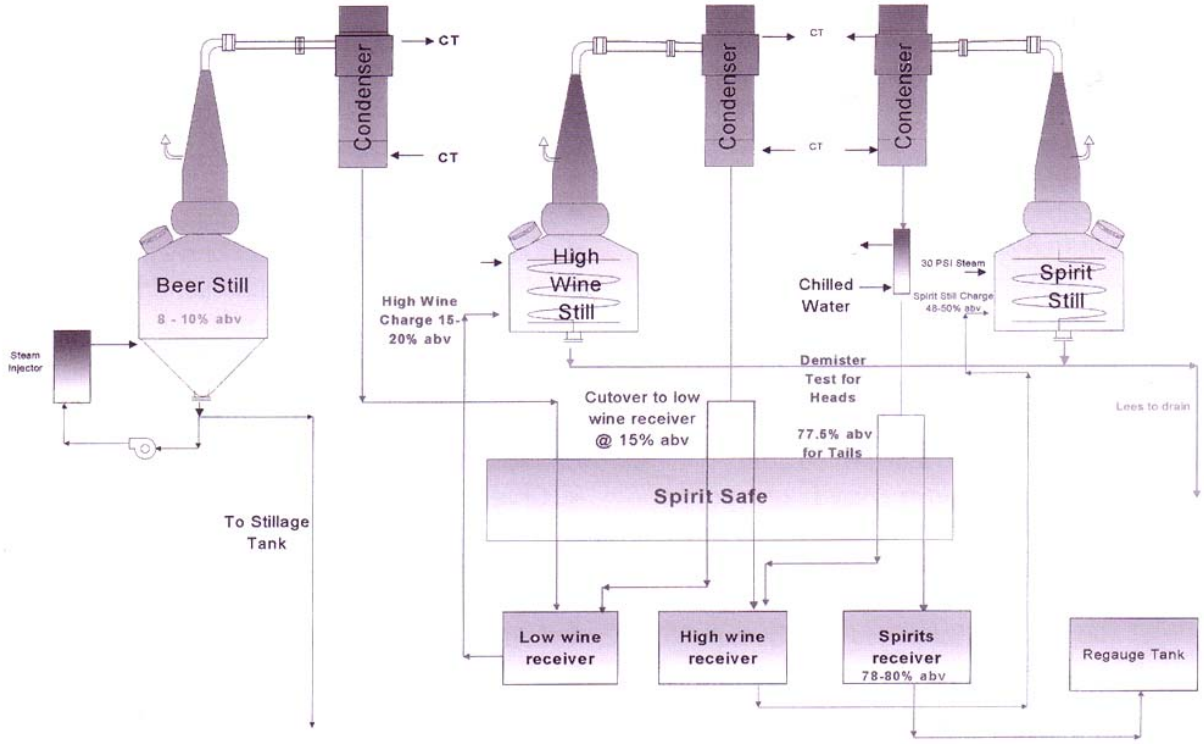


Figure 2.7: Diistillation apparatus diagram for a pot-stilled American bourbon whiskey (64).

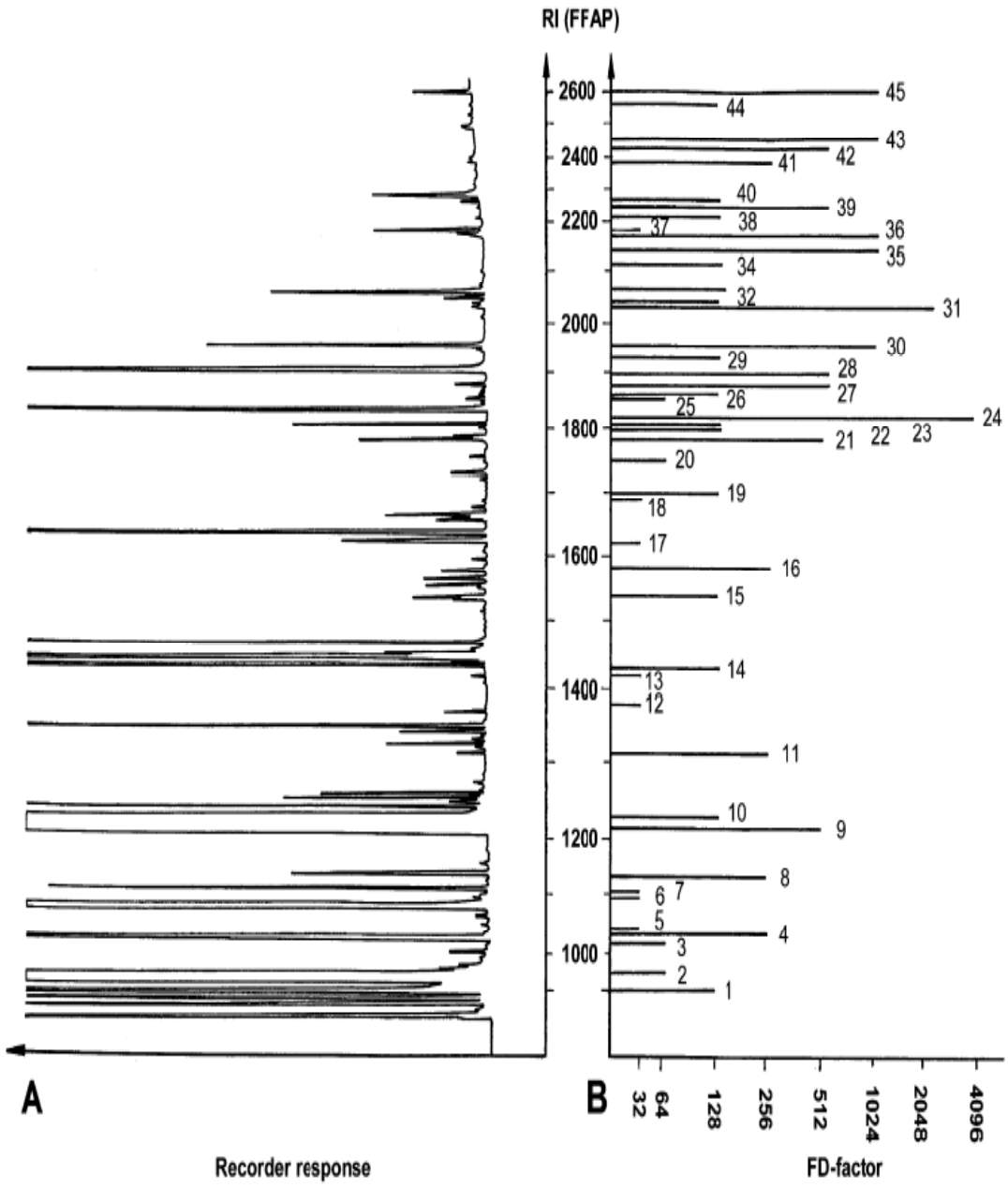


Figure 2.8: Gas chromatogram (left) and Flavor Dilution chromatogram (right) of a bourbon whiskey (3). Note the lack of regular correspondence between abundance (on the left) and dilution factor (on the right).

odorant[b]	odor quality[c]	fract.[d]	RI[e] on FFAP	RI[e] on DB-5	RI[e] on DB-1701	FD factor
1,1-diethoxyethane[f]	fruity	A	900	730	<800	128
ethyl 2-methylpropanoate	fruity	A	958	756	811	64
ethyl butanoate	fruity	A	1029	805	857	64
ethyl (*S*)-2-methylbutanoate	fruity	A	1040	850	900	256
ethyl 3-methylbutanoate	fruity	A	1049	849	906	32
2-methylpropan-1-ol	malty	C	1091	648	n.d.	32
unknown	fruity	B	1102	988	n.d.	32
3-methylbutyl acetate	fruity	B	1120	879	948	256
3-methylbutan-1-ol	malty	C	1215	735	844	512
ethyl hexanoate	fruity	B	1218	1000	1062	128
(*E*)-2-heptenal	fatty, green	B	1311	959	1063	256
nonanal	soapy	B	1380	1100	n.d.	32
2-isopropyl-3-methoxypyrazine	earthy	C	1413	1091	1145	32
ethyl octanoate	fruity	B + C	1420	1197	1263	128
(*E*)-2-nonenal	green	C + D	1533	1159	1275	128
(*E*,*Z*)-2,6-nonadienal[f]	green	C + D	1578	1153	n.d.	256
(*E*)-2-decenal	fatty	D	1624	1260	1370	32
(*E*,*E*)-2,4-nonadienal	fatty	D	1691	1211	n.d.	32
ethyl 2-phenylacetate	flowery	D	1695	n.d.	n.d.	128
4-methylacetophenone	sweet, almond-like		1747	1186	1326	64
α-damascone[f]	cooked apple	C	1779	1389	n.d.	512
(*E*,*E*)-2,4-decadienal	fatty	B + C	1791	1315	1447	128
2-phenylethyl acetate	flowery	B	1804	1256	1371	128
(*E*)-β-damascenone	cooked apple	B	1813	1389	1487	4096
2-methoxyphenol	phenolic	C	1849	1089	1225	64
2-phenylethyl propanoate	fruity		1860	n.d.	n.d.	128
(3*S*,4*R*)-*trans*-whiskylactone	coconut-like	C	1866	1295	1487	512
2-phenylethanol	flowery	D	1900	1113	1273	512
β-ionone	violet-like	C	1929	1495	1620	128
(3*S*,4*S*)-*cis*-whiskylactone	coconut-like	C + D	1946	1328	1522	1024
(*R*/*S*)-γ-nonalactone	coconut-like	D	2018	1365	1566	2048
4-ethyl-2-methoxyphenol	phenolic, clove-like	D	2030	1284	1413	128
δ-nonalactone	peach-like	D	2047	n.d.	1612	256
trans ethyl cinnamate	fruity	D	2108	1461	n.d.	256
γ-decalactone	peach-like	D	2132	1471	1690	1024
4-allyl-2-methoxyphenol	clove-like	C	2161	1357	1498	1024
4-ethylphenol	phenolic	C	2168	1168	1395	32
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[f]	seasoning-like	D	2206	1100	1362	128
unknown	coconut-like	D	2243	1562	1805	512
unknown	fruity	D	2265	n.d.	n.d.	128
γ-dodecalactone	peach-like	D	2381	1685	1900	256
(*Z*)-6-dodeceno-γ-lactone	peach-like	D	2425	1680	n.d.	512
unknown	flowery	A + E	2442	n.d.	n.d.	1024
2-phenylacetic acid	flowery	E	2552	1275	n.d.	256
4-hydroxy-3-methoxy-benzaldehyde	vanilla-like	D	2600	1400	1632	1024

Figure 2.9: Important odorants in a bourbon whiskey, identified by GCO and Aroma Extract Dilution Analysis (AEDA) (3). The authors identified odorants based on the following criteria: (i) retention indices on the capillary columns noted in the table, (ii) mass spectra obtained by MS-CI and MS-EI, and (iii) odor quality and threshold as determined by GCO.

compound	concentration [μg/L][a]	standard deviation [%][b]
ethanol	316000000	
3-methylbutanol	1060000	2
1,1-diethoxyethane	15300	1
2-phenylethanol	13900	2
ethyl octanoate	8340	9
3-methylbutyl acetate	2590	7
(3*S*,4*S*)-*cis*-whiskylactone	2490	6
4-hydroxy-3-methoxybenzaldehyde	2130	2
ethyl hexanoate	1990	1
2-phenylethyl acetate	1940	2
ethyl propanoate	793	4
ethyl butanoate	551	5
3-methylbutanal	342	10
(3*S*,4*R*)-*trans*-whiskylactone	337	8
4-allyl-2-methoxyphenol	240	1
2-methylpropanal	233	9
ethyl 2-methylpropanoate	134	10
γ-nonalactone	120	10
4-ethyl-2-methoxyphenol	59	3
2-methoxyphenol	56	7
ethyl 3-methylbutanoate	52	6
(*E*,*E*)-2,4-decadienal	39	6
2,3-butandione	33	10
(*S*)-ethyl 2-methylbutanoate	30	10
(*E*)-damascenone	11	5
(*E*)-2-nonenal	9	5
(*E*,*E*)-2,4-nonadienal	2.4	10
(*E*)-2-decenal	1.8	3
trans-ethyl cinnamate	1.7	10
γ-decalactone	1.6	10
(*E*,*Z*)-2,6-nonadienal	0.9	10

[a] The mean value obtained by analyzing three different samples taken from the same bottle. [b] The standard deviation of the mean value [%].

Figure 2.10: Absolute concentrations of important odorants in a bourbon whiskey, as calculated using Stable Isotope Dilution Analysis (SIDA) (4).

--3. Identification of Potent Odorants in Rye Whiskey--

I. JUSTIFICATION OF RESEARCH AND OBJECTIVES

Despite their economic importance, the aroma profiles of American whiskeys (both bourbon and rye) remain largely unexplored, with some important exceptions (3, 4, 35). The two predominant types of whiskey currently produced in the United States – rye and bourbon whiskeys – are considered to be distinct products, with unique flavor profiles, despite production protocols whose only difference is the percentage of rye or corn cereal grains, respectively, in the pre-distillation mash. Since wood-aging is generally thought to be responsible for much of the flavor of whiskey (5, 15), it is not necessarily clear that rye and bourbon should have significantly different flavor profiles.

The aroma profile of bourbon whiskey and the compounds which contribute significantly to it have recently been detailed (3) and verified by sensory assay (4). The objective of the present research was to similarly profile the aroma of rye whiskey and catalog the high-impact aroma compounds which contribute to this aroma profile. This information can then be compared to the literature information currently available on bourbon aroma, and conclusions can potentially be drawn about the relative uniqueness of rye whiskey.

Aroma profiles were obtained using extractive and non-extractive methods, combined with gas chromatography-olfactometry and gas chromatography-mass spectrometry in order to identify individual odorants and the aroma properties of those odorants. The extractive methods were based on liquid-liquid extraction, in order to both exclude as much ethanol as possible from the extract (40) and to obtain the most representative extract (41).

At the same time, because *all* extractive methods introduce a bias based on partitioning, this research also developed a method of direct injection of rye whiskey into a gas chromatograph, using appropriate, state-of-the-art inlet technology in order to achieve acceptable chromatography despite the large volume of water and low concentration of the compounds of interest (44, 47). After

determining that no appreciable bias was introduced from this injection technique, the aroma profiles of rye whiskey as determined by extractive and non-extractive techniques were compared.

II. MATERIALS AND METHODS

Materials

Four different commercial rye whiskeys, which are widely available nationally, were purchased at a local liquor store (Savoy, IL). Three of the four whiskeys – Jim Beam Rye, Old Overholt, and Rittenhouse – had a reported ethanol content by volume of 40%; the fourth whiskey – Wild Turkey Rye – had a reported ethanol v/v of 50.5%. Each was declared to be a straight rye whiskey according to government specifications (2). Mention of the brand names of these whiskeys does not imply any research contact or sponsorship, and is not for advertisement or endorsement purposes.

All authentic standards except for β-damascenone were of ≥98% purity; β-damascenone had a measured purity of 95%. Authentic standards were obtained from the following sources. Sigma-Aldrich (St Louis, MO): ethyl butyrate, ethyl caproate (hexanoate), ethyl propanoate, butyric acid, ethyl isovalerate, phenylacetic acid, ethyl isobutyrate, phenethyl acetate, 4-ethylphenol, *p*-cresol, γ-nonalactone, eugenol, 2,6-dimethoxyphenol, isovaleric acid, syringaldehyde, *cis*+*trans*-whiskey lactone (52.2% *trans*), β-ionone, guaiacol, 2-phenylethanol. Alfa Aesar (Lancaster, UK): ethyl cinnamate, 4-ethyl-2-methoxyphenol, ethyl vanillate. Avocado (Lancaster, UK): 4-vinyl-2-methoxyphenol. Baker (Phillipsburg, NJ): 2-methyl-1-propanol. Firmenich (Switzerland): β-damascenone. Fisher (Fair Lawn, NJ): 3-methyl-1-butanol. Fluka (Switzerland): vanillin, isoamyl acetate.

Dichloromethane, methanol, diethyl ether, and anhydrous sodium sulfate were purchased from Fisher Scientific (Fair Lawn, NJ).

Aroma Extraction

Liquid-Liquid Extraction: Aroma extracts were obtained by two methods: direct liquid-liquid extraction (LLX) and continuous liquid-liquid extraction (CLLX). CLLX is meant to be an exhaustive method of extraction (39).

For CLLX, a continuous extraction apparatus (Part # Z562440; Sigma Aldrich, St Louis, MO) with a condenser (7-inch length, 24/40 ground joints) at 0 ℃ was set up with 100 mL of dichloromethane in the receiving flask (250 mL, 24/40 ground glass joint) (CH_2Cl_2) as the solvent, and 250 mL of rye whiskey combined with 250 mL of deodorized water in order to reach 20% abv (%v/v) (except in the case of Wild Turkey, at 50.5% abv, which was combined at 200 mL whiskey to 300 mL deodorized water). Dichloromethane was refluxed through the whiskey for 18-24 hours (figure 3.1). The dichloromethane fraction was collected, dried over anhydrous sodium sulfate (Na_2SO_4), concentrated to 10 mL under a gentle nitrogen stream, and reserved for further analysis. Sample extracts were stored in a conventional freezer at approximately -18 ℃.

For LLX, whiskey was diluted to 20% abv (equal parts dilution with deodorized water for whiskeys with 40% abv, 2:3 dilution for Wild Turkey, which is 50.5% abv). Dilute whiskey (450 mL) was extracted with 45 mL of dichloromethane (3X) by shaking. The dichloromethane fractions from each whiskey were combined and dried over anhydrous sodium sulfate, concentrated to 10 mL under a gentle nitrogen stream, and reserved for further analysis. Samples were stored in a conventional freezer at approximately -18 ℃.

Solvent Assisted Flavor Evaporation (SAFE): SAFE is a gentle distillation technique, conducted under high vacuum, which is meant to separate an aroma extract into volatile and non-volatile components. SAFE is useful in order to present a clean sample for on-column injection techniques and to avoid artifact formation in split/splitless injections. LLX samples were subjected to SAFE according to the technique detailed by Song *et al* (78), based on the method detailed by Engel *et al* (54). The SAFE apparatus was similar to that described by Engel (54), consisting of a high-vacuum pump, a turbo-pump,

a receiving trap, and a waste trap. SAFE was conducted at extremely low pressure (approximately 10^{-5} torr) and very low temperature (-196 ℃) in order to avoid artifact formation and to trap volatile substances. SAFE was carried out for 2 hours.

Identification of Odorants

GCO Parameters: GCO was conducted using an Agilent 6890 Gas Chromatograph (Agilent, Santa Clara, CA), equipped with a Flame Ionization Detector and an Olfactometry Port (OD2; Gerstel, Mülheim an der Ruhr, Germany). For polar separations, a Restek RTX-Wax column (Restek, Bellefonte, PA) of 15 m length, 0.53 mm internal diameter, and 1 μm internal film was used. For non-polar separation, a Restek RTX-5 column of the same dimensions (15 m, 0.53 mm diameter, 1 μm film) was used. A Gerstel CIS-4 Programmable Temperature Vaporizer (PTV) inlet (Gerstel, Mülheim an der Ruhr, Germany) was used for all injections; for on-column injections, an on-column adapter for the CIS-4 was used.

Total runtime was 38.5 minutes. The linear velocity of the column was 22.4 m/min, with helium as the carrier gas. The flow was split post-separation through deactivated, uncoated fused silica tubing to a Flame Ionization Detector (FID) operating at 250 ℃, and an Olfactory Detection Port (ODP), with the transfer line held at 250 ℃. The CIS-4 inlet was programmed to hold at -50 ℃ until injection and for 0.10 minutes afterward. After that point, the initial inlet ramp was 10 ℃/s for 10 minutes, to a final temperature of 260 ℃. The oven was set to an initial temperature of 40 ℃, held for 5 minutes, after which it was ramped at a rate of 10 ℃/min to a final temperature of 225 ℃, at 38.5 minutes.

GC-MS Parameters: Gas Chromatography-Mass Spectrometry system consisted of an Agilent 6890 GC attached to a Hewlett-Packard mass spectrometer model 5973 set up for Electron Ionization (EI) at 70 eV. The column used for polar separations was a Restek RTX-Wax column of 30 m length, 0.25 mm internal diameter, and 0.25 μm film coating. Non-polar separation was by a Restek RTX-5 column of the same dimensions (30 m length, 0.25 mm internal diameter, 0.25 μm film). Spectra were compared

against the National Institute of Science and Technology (NIST) database. A Gerstel CIS-4 PTV inlet was used for split/splitless injections.

Total runtime was 72.5 minutes. The carrier gas was helium, and total flow through the column was 1.0 mL/min. The entire output was directed to the mass spectrometer, which was set up with a 3.80 min solvent delay and run in scan mode. The CIS-4 inlet was held at -50 ℃ until injection and for 0.10 minutes afterwards, at which point it ramped at 12 ℃/s to a final temperature of 250 ℃. The oven began at 35 ℃, at which it was held for 5 minutes, after which it was ramped to a final temperature of 225 ℃ at a rate of 4 ℃/min.

Identification of Compounds: Aliquots of undiluted whiskey, CLLX samples, LLX samples, and LLX samples post-SAFE were subjected to GCO analysis using cold-split and cold-splitless injections on a polar column (RTX-Wax). Retention times (RTs), odor impressions, and approximate intensities for each peak were recorded by the assessor. Aliquots of each sample were simultaneously subjected to GC-MS analysis on two phases (RTX-Wax and RTX-5).

Retention Indices (RIs) were calculated using the RTs of a series of standard alkanes of length C6 to C30, using the following equation:

$$RI = (RI_N - RI_n)\frac{RT - RT_n}{RT_N - RT_n},$$

where the subscript *n* indicates the standard alkane with an RT immediately preceding that of the target compound, and the subscript *N* indicates the standard alkane with an RT immediately following that of the target compound. Each alkane is assigned, by definition, an RI equivalent to its chain length multiplied by 100 (i.e.,).

The RI and sensory impression obtained from GCO were used to tentatively identify each peak. These tentative identifications were correlated, by RI, with spectral data from the GC-MS. Identification was confirmed by comparing identifications from the polar (RTX-Wax) column with those from the non-polar (RTX-5) column. Authentic standards for each tentative identification were analyzed using GCO.

An RI match on two phases (RTX-Wax and RTX-5) and a match using spectral data from GC-MS with an authentic standard was considered positive identification for that compound.

Dilution Analysis

Aroma Extract Dilution Analysis (AEDA): The aroma extract from Wild Turkey was selected for further analysis by dilution. For AEDA, the extract obtained from LLX was diluted stepwise in 1:3 dilutions using dichloromethane. A total of 10 dilutions (at a dilution factor of 1:59049) were made. Each diluted extract was analyzed by GCO. Flavor Dilution (FD) factors were calculated as the number of dilutions until a certain peak (and therefore compound) was no longer detectable by GCO (48).

Sample Dilution Analysis (SDA): Wild Turkey was selected for SDA in order to compare results with AEDA. Undiluted Wild Turkey whiskey, at 50.5% abv, was diluted stepwise in 1:3 increments with pure ethyl alcohol to a total of six dilutions (a dilution factor of 1:729). Flavor Dilution (FD) factors were calculated as the number of dilutions until a certain peak (and therefore compound) was no longer detectable by olfactometry (48).

Injection Method Comparison

A standard mix of seven different compounds was prepared in order to test the inlet bias resulting from the use of cold-splitless injection instead of cool, on-column injection (43). The compounds used were 3-methyl-1-butanol, guaiacol, 2-phenylethanol, cis-whiskey lactone, trans-whiskey lactone, syringol, and vanillin. These compounds represent a range of moieties, polarities, molecular weights, and volatilities; they are also known to be important to the characteristic aroma of whiskey. Compounds were prepared in 10 ppm and 100 ppm solutions in diethyl ether. The compound 3-methyl-1-butanol, which is known to be relatively unaffected by inlet bias due to its stability, volatility, and low molecular weight, was used as an internal standard.

Three injection protocols were compared: hot-splitless, cold-splitless, and cool, on-column. For hot-splitless, the inlet was maintained at 250 ℃, with the purge vent opened at 1 minute after injection.

For cold-splitless, the inlet was maintained at -50 ℃ until injection, at which point it was ramped to 250 ℃ in 1 minute, after which the purge vent was opened. For cool, on-column injection the inlet began at 40 ℃ and used oven tracking throughout the GC run. Both concentrations of standard solutions were run in duplicate for statistical verification. Relative abundance of each compound was calculated normalized against 3-methyl-1-butanol.

III. RESULTS

Injection Method Comparison

The examination of inlet bias showed no significant bias introduced by use of cold-splitless injection. While the differences were not significant, cold-splitless outperformed both other methods for all compounds at the 100 ppm concentration (see figure 3.2). At the lower 10 ppm concentration, there were differences between cool, on-column injection and cold-splitless injection for the two heaviest (least volatile) compounds used in this assay: vanillin and syringol. These differences, however, were considered unlikely to introduce bias into any dilution analysis, as the difference in levels detected was far lower than 1:3 (a single dilution factor) (figure 3.2).

Identification of Odorants

In direct GCO comparisons, no significant difference was found between LLX and CLLX extracts. The same peaks were identified in both. By GCO and GC-MS analysis, SAFE was shown to reduce the total intensity of odorant peaks and, in the case of syringaldehyde, an odorant with very low volatility, to completely eliminate the peak from the SAFE extract. Therefore, CLLX was determined to be unnecessary for obtaining a representative extract, while SAFE was determined to be potentially detrimental to the final extract.

A total of 40 aroma peaks were identified by GCO analysis of the LLX extract; 24 aroma peaks were identified by GCO analysis of the unextracted whiskey (table 3.1). The identified compounds were

mostly phenolic and lignin-pyrrolysis and -decomposition compounds from oak barrel aging (35), fusel alcohols from yeast fermentation, organic acids, esters, lactones, and norisoprenoids.

Sixteen additional peaks were identified in the LLX extract of whiskey, of which 7 peaks were identifiable (ethyl propanoate, ethyl isobutyrate, ethyl butyrate, ethyl hexanoate, isoamyl acetate, syringaldehyde, and γ-nonalactone), while 9 were not identifiable by odor impression and RI, and could not be located using GC-MS. In the case of γ-nonalactone, which nearly co-elutes with 4-ethyl-2-methoxyphenol on polar columns, it is possible that the impression for 4-ethyl-2-methoxyphenol alone in direct whiskey analysis is in fact an impression for both compounds, which were undistinguishable (by GCO) in the undiluted whiskey. It is alternatively possible that 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, which also elutes at the same RI on polar columns, is responsible for some of the odor impression listed for the other two compounds, since the impression recorded, that of a burnt-sugar, strawberry note, is very similar to that compound's odor impression. It was, however, not possible to detect 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone by GC-MS analysis of the whiskey or whiskey extract.

Dilution Analysis

Sample Dilution Analysis: The most persistent odorants in SDA – 2-phenylethanol, 3-methyl-1-butanol, and vanillin – lasted through 6 dilutions (FD = 6). Other potent odorants identified by SDA (FD = 5) were guaiacol, syringol, *cis*-whiskey lactone, 4-ethyl-2-methoxyphenol, *p*-vinylguaiacol, and β-ionone. All 24 odorants identified through direct GCO analysis of whiskey persisted through at least one dilution (24 odorants with FD ≥ 1) (table 3.1).

Aroma Extract Dilution Analysis: The most persistent odorant in AEDA (FD = 10) was guaiacol. Other potent odorants were 2-phenylethanol and syringol (FD = 9), 3-methyl-1-butanol and vanillin (FD = 8), *cis*-whiskey lactone (FD = 7), and β-damascenone and eugenol (FD = 6). All peaks identified in the GCO analysis of the LLX extract persisted for at least 3 dilutions in AEDA (table 3.1).

IV. DISCUSSION

All of the 31 compounds which were positively identified (either through LLX or direct analysis of whiskey) were previously known volatile components of whiskeys (3, 35, 47). It is possible that some of the unidentified odor peaks with unusual aroma attributes and moderate strength are important contributors to whiskey aroma – this can be tested by constructing whiskey models without accounting for these compounds and conducting sensory assays on them. While this will not identify these compounds, it could determine whether it is necessary to identify them in order to construct an accurate rye whiskey aroma model.

The majority of the compounds identified solely through analysis of the extract – that is, compounds for which an aroma peak was not identified in GCO analysis of the unextracted whiskey – were ethyl esters (4 of 7 identifiable peaks) and an acetate (1 of 7 identifiable peaks). This is worth noting because it is possible that this is a demonstration of extraction bias. These esters, which are short-chain and thus extremely soluble in a 40% ethanol-water matrix, are much less polar than their alcohol or acid precursors. Dichloromethane selects strongly against polar compounds (like short chain alcohols and acids), and so it is likely that esters were overextracted into dichloromethane; that is, they were in higher relative abundance in the extract than they are likely to be in the unextracted whiskey.

Considering the results of the two dilution analyses gives more credence to this theory. In general the two analyses – SDA and AEDA – provide quite similar results (table 3.1). Each method identifies a set of key potent odorants that is roughly equivalent, with a difference of one or two compounds among the key odorants. All 24 odorants identified by SDA are also important in AEDA. The order of importance of compounds, however, differs slightly in ways that support the extraction bias hypothesis.

For example, while 2-phenylethanol and 3-methyl-1-butanol are important odorants in both AEDA and SDA, they are both less important in AEDA than SDA. Both compounds are fusel alcohols, and dichloromethane, the solvent used for LLX, is known to select against alcohols. At the same time, ethyl

propanoate, a short-chain ethyl ester, was found to be a relatively important odorant in AEDA (FD = 5), but was not identified at all through SDA. Dichloromethane selects for non-polar compounds like ethyl propanoate – it therefore is sensible to think that its importance in AEDA might be exaggerated by this solvent bias.

Despite these potential biases, the agreement between AEDA and SDA was significant. AEDA is something of a gold standard for aroma analysis; despite its imperfections it is widely used and its advantages and limitations are well understood (3, 4, 37, 40, 48-50, 65). Thus, the ability of SDA to identify 85% of the odorants identified through AEDA – including *all* of the key odorants (FD = 6) identified through AEDA – validates it as a potential alternative to AEDA.

SDA has several advantages over AEDA for the analysis of distilled alcoholic beverages. First, because it does not require sample preparation, it is rapid and it eliminates the need for potentially environmentally damaging solvents. Both of these qualities make SDA potentially more suitable for industry use. Finally, SDA eliminates the potential for extraction bias. Theoretically this should provide an advantage in rapidly identifying important odorants in any distilled alcoholic beverage without the immediate need to verify by quantification and calculation of odor activity values (OAVs), a process which can involve difficult and time-consuming work determining thresholds and synthesizing isotopically labeled internal standards for quantification. However, in order to explore whether SDA indeed offers this, it must first be checked against just this sort of verification, a process which is explored in the next chapter.

V. FIGURES AND TABLES

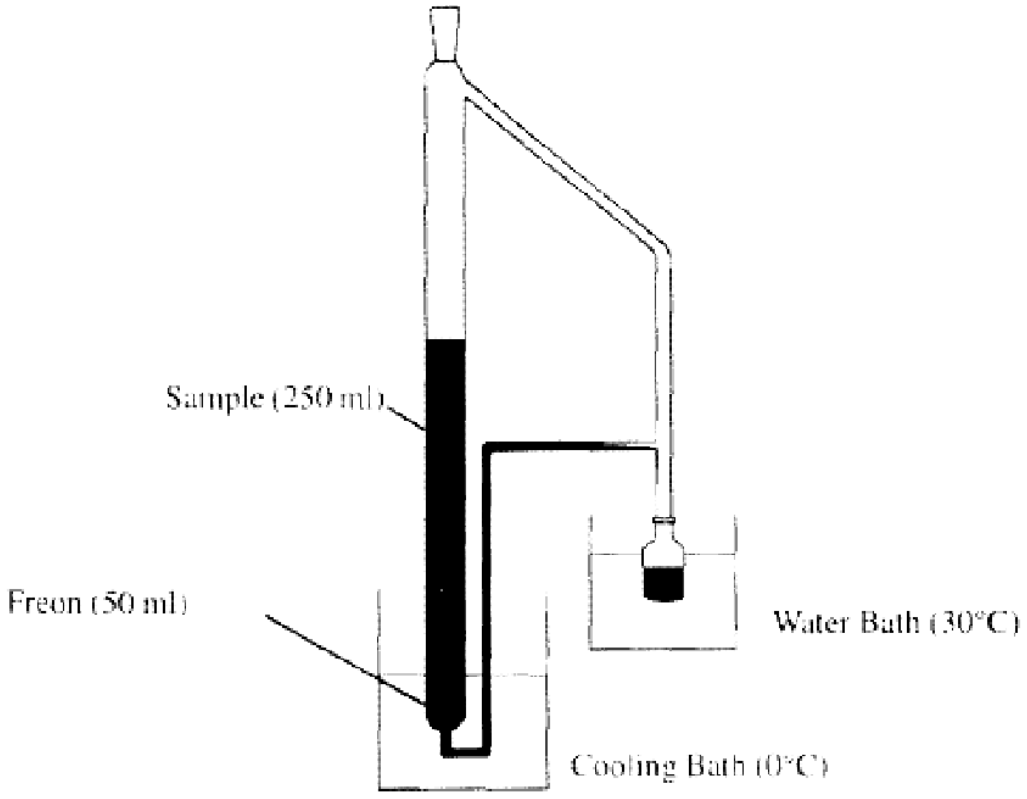


Figure 3.1: Schematic for Continuous Liquid-Liquid Extraction (CLLX), using Freon. For dichloromethane, the set-up is the same but the second (0 °C) cooling bath is unnecessary. Not noted in the diagram is the condenser at the top of the apparatus, which causes the solvent to condense and drip through the liquid, performing many microextractions quickly (39).

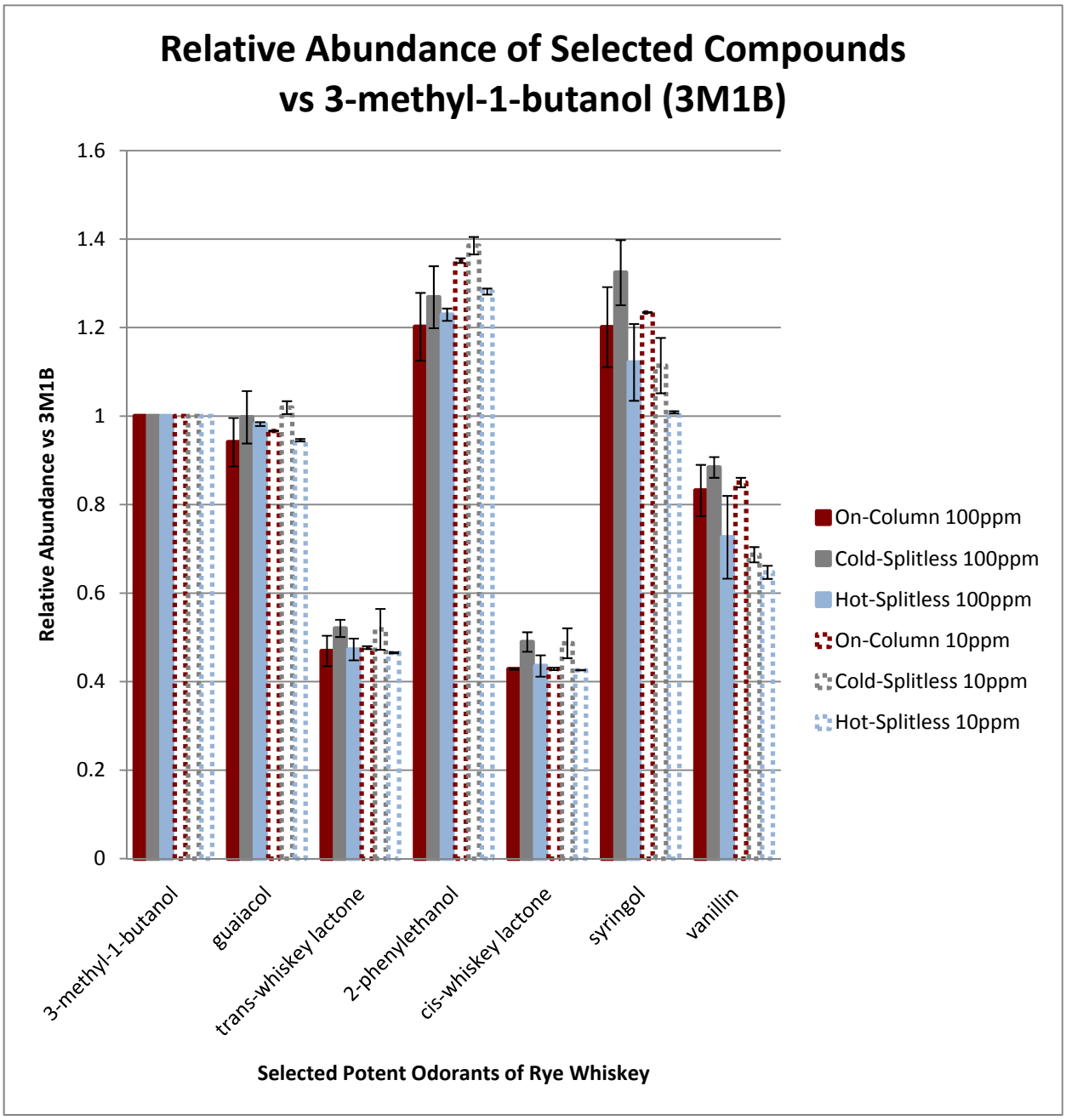


Figure 3.2: Inlet bias comparison results. All peak areas are normalized against the corresponding area of 3-methyl-1-butanol, in order to facilitate comparison of results across injection type.

Table 3.1: Important Odorants Identified in Rye Whiskey Extracts and by Direct Injection of Rye Whiskey

Compound	Aroma Impression	LLX	Direct	RI-Wax	RI-5	FD (AEDA)	FD (SDA)
guaiacol	smoke, sweet	X	X	1851	1092	10	5
2,6-dimethoxyphenol	spicy, smoky, vanilla	X	X	2247	1355	9	5
phenylethyl alcohol	floral, rosy	X	X	1899	1133	9	6
3-methyl-1-butanol	fusel, almond, chocolate	X	X	1230	835	8	6
vanillin	marshmallow	X	X	2531	1403	8	6
cis-whiskey lactone	coconut	X	X	1941	1325	7	5
eugenol	spicy	X	X	2155	1357	6	2
β-damascenone	apple	X	X	1816	1381	6	4
ethyl propanoate	fake fruit, marker	X		924	725	5	
isovaleric acid	cheesy, fecal	X	X	1663	918	5	4
phenylacetic acid	bad rose	X	X	2546	1424	5	3
phenylethyl acetate	floral	X	X	1809	1256	5	2
2-methyl-1-propanol	damp, fusel?	X	X	1133		4	4
4-ethyl-2-methoxy-phenol	spicy, sugar, berry[a]	X	X	2018	1279	4	5
ethyl isobutyrate	fake berry, sharp	X		970	773	4	
ethyl vanillate	spicy, bad, cinnamon	X	X	2604	1587	4	4
p-vinylguaiacol	curry, ruinous	X	X	2172	1315	4	5
syringaldehyde	woody, smoky, vanilla	X		2857	1675	4	
4-ethylphenol	bandage	X	X	2167	1182	3	3
acetic acid	vinegar	X	X	1448	816	3	3
ethyl butyrate	fake fruit, maker	X		1038	820	3	
ethyl cinnamate	fruity, fake	X	X	2123		3	2
ethyl hexanoate	fake fruit, sharp, sweat, marker	X		1244	1008	3	
ethyl isovalerate	sharp, fake fruit	X	X	1064	870	3	4
trans-whiskey lactone	coconut, stale	X	X	1880	1290	3	1
unknown	bug, bitter, green	X		1431		3	
cis-6-dodecenyl-γ-lactone[b]	cheesy, herb, cilantro	X		2377		3	
unknown	marker	X		1424		3	
unknown	nutty, fatty, buttery	X		1314		3	
unknown	vanilla	X	X	2495		3	2
unknown	waxy, cilantro, cucumber	X		1504		3	
β-ionone	violet	X	X	1901	1425	3	5
butyric acid	cheesy	X	X	1621	865	2	2
isoamyl acetate	fake banana	X		1132	894	2	
unknown	buttery, cooked milk	X		1675		2	
unknown	choking, acrid	X		2265		2	
unknown	citrus, green, waxy?	X		1543		2	
unknown	cucumber, sweet	X		1583		2	
γ-nonalactone	creamy, peach, strawberry[a]	X		2011	1361	2	
p-cresol	fecal, bandage	X	X	2069	1106	1	3

[a]It is possible that this odor impression is, at least partly, due to the co-elution of 4-hydroxy-2,5-dimethyl-3(2H)-furanone, which is known to have a low enough threshold in air so as to be detectable in GCO but not by GC-MS assays. [b]This compound is *tentatively* identified by RI match and odor description; it was not possible to find a positive spectrum match in GC-MS so as to identify it on two phases.

--4. Quantification Of Potent Odorants In Rye Whiskey—

I. JUSTIFICATION OF RESEARCH AND OBJECTIVES

In the previous research (detailed in chapter 3), the key potent odorants in American rye whiskey were identified by two different dilution analysis techniques: Sample Dilution Analysis (SDA), a non-extractive, direct-injection method, and Aroma Extract Dilution Analysis (AEDA), a method which relies on a solvent extract obtained through Liquid-Liquid Extraction (LLX). AEDA is a widely used and accepted technique, while SDA is a novel technique developed for this research. The previous research showed that the results obtained through each method were substantially similar. The current research is proposed in order to further verify the validity of the results provided by SDA.

Identifying key odorants is important for a qualitative approach to determining a product's odor profile: that is, identifying key odorants is important both for contributing to the extant body of knowledge and for indicating which components may be important for optimization of a product's flavor profile. Key-odorant identification, however, should be thought of as only one step in a process of determining exactly which compounds and combinations of compounds are most important to determining the aroma perception of a product. In order to further elucidate the role of each important compound, it is necessary to quantify them.

Quantification is a necessary intermediate step for the construction of aroma models, which are the best verification of an accurate elucidation of a product's sensory aroma profile. Furthermore, quantification data can be used to determine values like Odor Activity (OAV), a measurement of aroma significance based on a compound's presence above its threshold value in the product matrix.

In this study, quantification is performed through Stable Isotope Dilution Analysis (SIDA). In SIDA, an isotopically-labeled standard is synthesized (or obtained from previous research or commercial sources) for each compound of interest, usually with Carbon-13 or deuterium (Hydrogen-2) labeling. These labeled analogues' physical and aroma properties are identical to those of the unlabeled

compounds, but the mass ions produced from these analogues under mass spectrometric analysis are distinct from those produced by the actual unlabeled compounds of interest. In a best-case scenario, the added molecular weight of the labeling causes physical separation by gas chromatography of the labeled analogue and the compound of interest, but this is not necessary for quantification, as modern chromatographic software allows the extraction of individual mass ion chromatographs simultaneously.

It is necessary to calculate "response factors" (R_f) for each compound and its labeled analogue. Response factors can be calculated by determining the linear relationship between the spectrometric area ratio of mass ions for the compound of interest and the labeled analogue and the actual mass ratio of each standard used. Then, in SIDA, the unknown mass of the compound of interest in the sample can be calculated, given the known mass of the labeled standard in the sample, the R_f, and the observable area ratio of mass ions in the GC-MS chromatograph of the sample.

SIDA relies on introducing a "spike" of labeled analogues into the sample before any extractive or analytical work is done. Because the physical properties of the labeled analogue are the same as those of the compound of interest, the mass ratio of the analogue and compound of interest should remain the same throughout analysis. It can be a challenge to properly incorporate the spike into some matrices – for example, cheese, a semi-solid, hydrophobic, fat and protein matrix – but rye whiskey provides no such difficulties. Methanol, diethyl ether, and dichloromethane, the three solvents used in this work for the preparation of spike solutions, are highly soluble in the ethanol-water matrix of whiskey, and the liquid phase makes even distribution and dispersion of the labeled compound a non-issue.

II. MATERIALS AND METHODS

Materials

Four different commercial rye whiskeys, which are widely available nationally, were purchased at a local liquor store (Savoy, IL). Three of the four whiskeys – Jim Beam Rye, Old Overholt, and

Rittenhouse – had a reported ethanol content by volume of 40%; the fourth whiskey – Wild Turkey Rye – had a reported ethanol v/v of 50.5%. Each was declared to be a straight rye whiskey according to government specifications (2). Mention of the brand names of these whiskeys does not imply any research contact or sponsorship, and is not for advertisement or endorsement purposes.

All authentic standards except for β-damascenone were of ≥98% purity; β-damascenone had a measured purity of 95.01%. Authentic standards were obtained from the following sources. Sigma-Aldrich (St Louis, MO): ethyl butyrate, ethyl caproate (hexanoate), ethyl propanoate, butyric acid, ethyl isovalerate, phenylacetic acid, ethyl isobutyrate, phenethyl acetate, 4-ethylphenol, p-cresol, γ-nonalactone, eugenol, 2,6-dimethoxyphenol, isovaleric acid, syringaldehyde, cis+trans-whiskey lactone (52.2% trans), β-ionone guaiacol, 2-phenylethanol. Alfa Aesar (Lancaster, UK): ethyl cinnamate, 4-ethyl-2-methoxyphenol, ethyl vanillate. Avocado (Lancaster, UK): 4-vinyl-2-methoxyphenol. Baker (Phillipsburg, NJ): 2-methyl-1-propanol. Firmenich (Switzerland): β-damascenone. Fisher (Fair Lawn, NJ): 3-methyl-1-butanol. Fluka (Switzerland): vanillin, isoamyl acetate.

Isotopically labeled standards were purchased as follows. CDN (Canada): d_3-acetic acid, d_7-butyric acid, d_{11}-ethyl hexanoate, d_3-*p*-cresol. Isotec (Miamisburg, OH): $^{13}C_2$-phenylacetic acid. Sigma-Aldrich (St. Louis, MO): d_5-ethanol.

Isotopically labeled standards not available for purchase were synthesized according to the following references or as detailed in the following Methods section (see figure 4.4): 2-[$^2H_3$]-methoxy-6-methoxyphenol (Appendix A (66)); 2-methyl-[2,3-$^2H_2$]-1-propanol (below (67)); 2-[1,2-$^{13}C_2$]-phenylethanol (68); 3-methyl-[3,4-$^2H_2$]-1-butanol (67); 4-[$^2H_5$]-ethyl-2-methoxyphenol (below (69)); 4-[$^2H_5$]-ethylphenol (below (69)); (*E*)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)-[1-$^2H_3$,3-$^2H_1$]-but-2-en-1-one ($^2H_4$-β-damascenone) (70); $^2H_3$-β-ionone (70); *cis*-/*trans*-β-methyl-γ-[3,4-$^2H_2$]-octanolide ($^2H_2$-(*cis*/*trans*)-whiskey lactone) (as below); ethyl [$^2H_7$]-butyrate (as below); [$^2H_5$]-ethyl cinmmatate (as below); ethyl [$^2H_{11}$]-hexanoate (as below); [$^2H_5$]-ethyl isobutyrate (as below); ethyl 3-methyl-[3,4-$^2H_2$]-

butyrate ($^2H_2$-ethyl isovalerate) (as below); [$^2H_5$]-ethyl vanillate (as below); 2-[$^2H_3$]-methoxy-4-(2-propenyl)-phenol ($^2H_3$-eugenol) (below and Appendix A (66, 71)); [3,4-$^2H_2$]-γ-nonalactone (72); 2-[$^2H_3$]-methoxyphenol ($^2H_3$-guaiacol) (Appendix A (66)); 3-methyl-[3,4-$^2H_2$]-butyl acetate (as below); 3-methyl-[3,4-$^2H_2$]-butyric acid ($^2H_2$-isovaleric acid) (67); [1,2-$^{13}C_2$]-phenylethyl acetate (as below); 4-vinyl-2-[$^2H_3$]-methoxyphenol ($^2H_3$-*p*-vinylguaiacol) (73); 4-hydroxy-3-[$^2H_3$]-methoxy-5-methoxybenzaldehyde ($^2H_3$-syringaldehyde) (below and Appendix A); 4-hydroxy-3-[$^2H_3$]-methoxybenzaldehyde ($^2H_3$-vanillin) (66).

Other reagents were purchased from the following companies. Fisher (Fair Lawn, NJ): dichloromethane, diethyl ether, sulfuric acid, anhydrous sodium sulfate, acetic acid, methanol, pentane, hydrochloric acid. US Silica Company (Berkeley Springs, WV): Florisil. Acros (New Jersey): ethanol. Aldrich (Milwaukee, WI): silica gel, *trans*-cinnamic acid. Sigma-Aldrich (St Louis, MO): isopropylmagnesium bromide, pentanal, acryloyl chloride, acetyl chloride, vanillic acid, triethyl amine, titanium isopropoxide, Grubb's Catalyst (Generation I), Wilkinson's Catalyst, tetrahydrofuran.

Synthesis of Labeled Standards

$^2H_2$-Whiskey Lactone: The synthesis of labeled whiskey lactone was based on the synthesis of massaiolactone as detailed by Gupta *et al* (72) (figure 4.1 and Appendix 3). First isopropenylmagnesium bromide in tetrahydrofuran (THF) (25 mmol) was nucleophilically added to pentanal (23 mmol) to form 2-methyl-1-hepten-3-ol (1) under nitrogen purge in an ice bath (0 ℃). After the reaction had gone to completion, saturated NH_4Cl was added, and the mixture was extracted with diethyl ether (Et_2O) to obtain a THF-Et_2O extract of 2-methyl-1-hepten-3-ol. The extract was dried over anhydrous sodium sulfate (Na_2SO_4) and the solvent was removed by evaporation through a Vigreaux Column and High Vacuum Transfer (HVT). Actual yield was 2.17 g (74% of theoretical yield) of 2-methyl-1-hepten-3-ol.

(2) The 2-methyl-1-hepten-3-ol was esterified with acryloyl chloride in order to yield 2-methyl-1-hepten-3-yl acryloylate. Under a nitrogen purge in an ice bath (0 ℃), 0.010 mol of 2-methyl-1-hepten-3-ol was dissolved in CH_2Cl_2 with 0.012 mol of triethylamine (Et_3N). With stirring, 0.012 mol of acryloyl

chloride was added to the mixture, and stirred for six hours. After the reaction was complete, deionized water was added and the CH_2Cl_2 was removed. The aqueous layer was extracted with Et_2O and the ether layer was combined with the dichloromethane layer. The combined CH_2Cl_2+Et_2O layer was washed with 10% H_2SO_4 and then dried over anhydrous Na_2SO_4. After HVT, the solvent was removed to yield the ester product, with a yield of 1.00 g (55% theoretical yield) of 2-methyl-1-hepten-3-yl acryloylate.

The unsaturated form of whiskey lactone (dehydro-whiskeylactone) was formed (3) using a ring closing by Grubb's Catalyst Generation I. Under a nitrogen purge with stirring, 0.002 mol of 2-methyl-1-hepten-3-yl acryloylate was dissolved in CH_2Cl_2 with 20% molar mass of Generation I Grubb's Catalyst (0.0004 mol) and 300 µL of titanium isopropoxide. The mixture was refluxed for 36 hours, then the condenser was switched with a Vigreaux column and the mixture was refluxed until most of the solvent was removed (the reaction mixture was reduced to a brown sludge). The reaction mixture was brought back up in diethyl ether, then passed through a packed Florisil column to remove the Grubb's Catalyst. The mixture was then neutralized with saturated Na_2CO_3 and NaCl and dried over anhydrous Na_2SO_4. The reaction mixture was then concentrated and passed through a packed silica gel column in order to remove any remaining ester. Finally, the solvent was evaporated to yield the final dehydro-lactone product, with a yield of 0.1565 g (51% of theoretical yield) of dehydro-whiskey lactone.

Finally, $^2H_2$-whiskey lactone (*cis-/trans*-β-methyl-γ-[3,4-$^2H_2$]-octanolide) was synthesized by deuteration of the unsaturated lactone using Wilkinson's Catalyst (4). In a pressure reactor, 0.114 g of dehydro-whiskey lactone and 30 mg of Wilkinson's catalyst were dissolved in deuterated methanol. A deuterium line was attached to the reactor, and, after venting to flush the vial, the vial was sealed and pressurized to 20 psi. The reaction proceeded for 72 hours. The product was extracted into Et_2O and purified by column chromatography using packed silica gel columns and a mobile phase of 90:10

pentane:Et_2O. Yield was 0.105 g (89.7% of theoretical yield) of $^{2}H_2$-*cis/trans*-whiskey lactone (52% *cis*, 48% *trans*).

Acetate Esters: $^{13}C_2$-phenethyl acetate and d_2-isoamyl acetate were synthesized with the same procedure (Appendix 2). The respective labeled alcohols were dissolved with a molar excess of triethylamine in dichloromethane under nitrogen and in an ice bath. Acetyl chloride, dissolved in dichloromethane, was added to the mixture, which was allowed to stir until the reaction was complete. The reaction was quenched by the addition of water, the CH_2Cl_2 layer was collected, and the aqueous layer was re-extracted with diethyl ether. The CH_2Cl_2+Et_2O fraction was neutralized with 10% H_2SO_4 and saturated $NaHCO_3$, and finally the solvent was removed to yield the final product.

Ethyl esters: d_5-ethyl cinnamate, d_5-ethyl vanillate, d_5-ethyl isobutyrate, and d_2-ethyl isovalerate were synthesized with the same procedure. Equal molar amounts of the precursor acid (unlabeled, in the case of vanillic acid, *trans*-cinnamic acid, and isobutyric acid; labeled, in the case of d_2-isovaleric acid) and d_5-ethanol (unlabeled ethanol, in the case of d_2-isovaleric acid) were combined in a small vial with 1 drop of sulfuric acid. The reaction mixture was incubated overnight (approximately 18 hours) at 60 ℃. After incubation, the reaction mixture was neutralized with Na_2CO_3, and extracted (3x) with diethyl ether. The Et_2O extract was dried with anhydrous Na_2SO_4, and solvent was removed with a gentle N_2 stream.

d_5-4-ethyl-2-methoxyphenol/d_5-4-ethylphenol: the synthesis of these labeled standards followed the procedure for the synthesis of d_3-4-ethyl-2-methoxyphenol/d_3-4-ethylphenol laid out by Rayne and Eggers (69), except that deuterium (instead of hydrogen) was used to sparge d_3-1-(4-hydroxyphenyl)ethanone and d_3-1-(4-hydroxy-3-methoxyphenyl)ethanone in order to yield d_5-4-ethylphenol and d_5-4-ethyl-2-methoxyphenol.

d_2-2-methyl-1-propanol: the synthesis of this labeled standard followed the synthesis for d2-3-methyl-1-butanol described by Steinhaus and Schieberle (67), but substituted 2-methyl-2-propen-1-ol for 3-methyl-3-buten-1-ol.

d_3-eugenol: this method references the work of Schneider and Rolando (66) and Kulkarni *et al* (71). First, in a flask equipped with a stir bar, reflux condenser, and N_2-purge, 10 mL of N,N-diethylaniline (79 mmol) was combined with 10.6 g (80 mmol) of aluminum chloride and dissolved in toluene. The mixture was stirred for 15 minutes, and then 6.56 eugenol (40 mmol) dissolved in 15 mL toluene was added dropwise. The mixture was refluxed for 2 hours at 108 ℃. The mixture was allowed to cool, and then added dropwise into 50 mL ice-cold water with stirring. The pH was adjusted to 1-2, and the mixture was transferred to a separatory funnel. The toluene layer was reserved, and the aqueous layer was extracted with diethyl ether (3 x 20 mL). The ether and toluene extracts were combined, and the solvent extract (ether+toluene) was washed with 2N HCl (2 x 20 mL). The solvent layer was back-extracted with 2N NaOH (3 x 15 mL), and the aqueous extract (brown-colored) was reserved and re-extracted with diethyl ether (3 x 25 mL) in order to remove the brown-colored product. The ether was dried with anhydrous Na_2SO_4 and then removed under N_2-purge to yield crude 4-allylcatecol, which was purified using flash chromatography (with a mobile phase of 1:1 pentane:diethyl ether) for a final yield of 5.32 g 4-allylcatecol.

Next, 1.5 g (10 mmol) of 4-allycatecol was transferred into a screw-top test tube with a stir bar and PTFE-lined cap, and dissolved in 10 mL ethanolic 2N NaOH. d_3-Iodomethane (1.45 g) was dissolved in 10 mL of ethanol, and, keeping a gentle N_2-purge on constantly, this solution was added dropwise to the 4-allylcatecol solution. The tube was purged with N_2 and shaken vigorously (with periodic venting). The mixture was left stirring for 72 hours, with occasional inversion of the test-tube to allow for complete reaction of the entire tube. The reaction mixture was then poured into a 500-mL beaker, and the ethanol was removed using a combination of N_2-purge and external warm-water bath. Water (150

mL) was used to solubilize and transfer the reaction mixture to a 250-mL separatory funnel, and the mixture was extracted with CH_2Cl_2 (4 x 20 mL) to remove the dimethoxylated (undesirable) product. The pH of the aqueous layer was then adjusted to <1, and extracted with CH_2Cl_2 to isolate the methoxyphenol product. The dichloromethane extract was backwashed with 1N NaOH (3 x 15 mL) in order to further isolate the product, and the aqueous layer was then adjusted to <1 pH with 4N HCl and re-extracted with diethyl ether in order to obtain the product. The ether layer was washed with water (2 x 10 mL) and dryed with anhydrous Na_2SO_4, reduced to approximately 10 mL in volume, and subjected to HVT. Finally, the ether layer was subjected to flash chromatography on silica gel (mobile phase 80:20 pentane:CH_2Cl_2) to obtain pure product. The dichloromethane was removed by evaporation, yielding d_3-4-allyl-2-methoxyphenol (d_3-eugenol), with a final yield of 0.1748 g.

d_3-syringaldehyde: first, 0.50 g (3 mmol) of of 3,4-dihydroxy-5-methoxybenzaldehyde was dissolved in 5 mL of aqueous 40% KOH in a screw-capped test tube (PTFE-top) with a N_2-purge and a stir bar. Then, over the course of 30 minutes (5-6 drops every 5 minutes), 0.42 g (3.2 mmol) of d_6-dimethylsulfate was added to the reaction tube. The vial was then capped and stirred for 2 hours, during which time the mixture became cloudy, brown/green, and viscous. The reaction was periodically checked for completion by removing 5-6 drops of mixture, adding to a vial containing 1 mL aqueous 1N HCl and 0.5 mL ethyl acetate, and analyzing the ethyl acetate layer by GC-MS. The reaction was continued, adding d_6-dimethylsulfate, until nearly all starting material had been consumed. Once the reaction was complete, the mixture was acidifed to pH 1-2, and extracted with ethyl acetate (1 x 10 mL, 4 x 5 mL). The ethyl acetate layer was washed with saturated NaCl and then dried over anhydrous Na_2SO_4. The solution was then filtered through glass wool and the solvent was removed using a combination of N2-purge and hot water bath. The final product was weighed for a final yield of 0.3194 g and resolubilized in diethyl ether for storage.

Characterization of Isotopic Standards (MS-EI)

d_3-guaiacol *m/z* (%): 109 (100), 127 (82), 81 (63), 53 (26), 52 (16), 51 (12), 50 (10)

d_3-2,6-dimethoxyphenol *m/z* (%): 157 (100), 96 (30), 142 (27), 139 (21), 114 (18), 39 (17), 95 (15), 51 (14), 93 (13), 68 (12), 65 (11), 111 (11), 53 (11), 158 (10)

d_2-2-methyl-1-propanol *m/z* (%): 45 (100), 44 (60), 43 (53), 42 (39), 40 (12), 41 (11)

$^{13}C_2$-2-phenylethanol *m/z* (%): 92 (100), 93 (56), 124 (26), 66 (13)

d_2-3-methyl-1-butanol *m/z* (%): 44 (100), 56 (92), 43 (81), 45 (72), 42 (59), 57 (52), 71 (50), 59 (32), 72 (28), 55 (25), 46 (21), 40 (18), 41 (18), 58 (12), 39 (11)

d_5-4-ethyl-2-methoxyphenol *m/z* (%): 139 (100), 157 (35), 140 (12), 124 (11), 96 (11)

d_5-4-ethylphenol *m/z* (%): 109 (100), 127 (27), 110 (10)

d_3-acetic acid *m/z* (%): 46 (100), 45 (58), 63 (41), 44 (11)

d_4-β-damascenone *m/z* (%): 73 (100), 121 (78), 45 (29), 105 (25), 91 (15), 41 (13), 120 (12), 77 (11), 194 (10)

d_3-β-ionone *m/z* (%): 180 (100), 46 (66), 91 (15), 41 (14), 181 (14), 93 (12), 77 (11), 138 (10)

d_7-butyric acid *m/z* (%): 63 (100), 77 (29), 46 (38), 44 (20), 50 (18), 45 (15)

d_2-*cis*-whiskey lactone *m/z* (%): 101 (100), 44 (47), 43 (29), 45 (28), 73 (26), 41 (25), 69 (25), 57 (24), 87 (20), 58 (16), 42 (16), 72 (11)

d_2-*trans*-whiskey lactone *m/z* (%): 101 (100), 44 (32), 45 (31), 73 (27), 43 (24), 41 (15), 87 (15), 69 (14), 57 (14), 42 (13), 86 (10)

d_7-ethyl butyrate *m/z* (%): 50 (100), 78 (90), 91 (43), 46 (34), 63 (15), 45 (15), 96 (13), 77 (11), 44 (10)

d_5-ethyl cinnamate *m/z* (%): 131 (100), 103 (49), 77 (34), 181 (29), 51 (23), 132 (19), 102 (15), 148 (14), 149 (12), 105 (11)

d_{11}-ethyl hexanoate *m/z* (%): 91 (100), 50 (78), 110 (52), 46 (43), 63 (35), 82 (28), 105 (24), 77 (18), 64 (17), 45 (15), 44 (14), 48 (12), 42 (11), 73 (11), 62 (10), 49 (10), 72 (10)

d_5-ethyl butyrate *m/z* (%): 43 (100), 41 (33), 71 (33), 42 (18), 121 (13)

d_2-ethyl isovalerate *m/z* (%): 88 (100), 59 (83), 87 (75), 60 (49), 43 (42), 42 (41), 61 (38), 45 (34), 89 (27), 70 (22), 44 (11), 40 (11), 41 (10)

d_5-ethyl vanillate *m/z* (%): 151 (100), 201 (33), 123 (17), 169 (15), 52 (11), 152 (11), 51 (10)

d_3-eugenol *m/z* (%): 167 (100), 149 (47), 103 (43), 77 (41), 131 (37), 91 (34), 55 (31), 104 (31), 133 (29), 140 (24), 51 (21), 39 (20), 121 (17), 65 (15), 168 (15), 78 (15), 150 (14), 63 (12), 132 (12), 53 (12), 105 (11), 94 (10), 66 (10)

d_2-γ-nonalactone *m/z* (%): 87 (100), 43 (11)

d_2-isoamyl acetate *m/z* (%): 43 (100), 72 (28), 56 (23), 57 (17), 71 (15), 42 (13), 45 (12), 44 (12)

d_2-isovaleric acid *m/z* (%): 60 (100), 45 (42), 43 (34), 42 (32), 61 (25), 40 (11), 44 (10), 46 (10)

d_3-*p*-cresol *m/z* (%): 111 (100), 110 (65), 109 (55), 82 (20), 79 (19), 53 (14), 83 (11), 54 (11), 81 (11), 55 (10), 80 (10), 52 (10)

$^{13}C_2$-phenethyl acetate *m/z* (%): 106 (100), 43 (61), 92 (19)

$^{13}C_2$-phenylacetic acid *m/z* (%): 92 (100), 138 (32), 91 (24), 93 (18), 66 (13), 65 (10), 39 (10)

d_3-*p*-vinylguaiacol *m/z* (%): 153 (100), 135 (83), 107 (40), 77 (35), 51 (16), 79 (13), 52 (12), 78 (12), 53 (12), 39 (10)

d_3-syringaldehyde *m/z* (%): 185 (100), 184 (64), 96 (17), 51 (16), 67 (15), 114 (14), 39 (13), 79 (12), 68 (11), 53 (11), 186 (11), 50 (11)

d_3-vanillin *m/z* (%): 154 (100), 155 (89), 81 (24), 109 (21), 52 (18), 51 (17), 53 (17), 126 (17), 50 (10), 79 (10)

Calibration of Stable Isotopes

Stable isotope standards were divided into six groups in order to avoid co-elution, so as to obtain the most accurate GC-MS results, as follows. Group 1: guaiacol/d_3-guaiacol, 4-ethyl-2-methoxyphenol/d_5-4-ethyl-2-methoxyphenol, *p*-cresol/d_3-*p*-cresol, 4-ethylphenol/d_5-4-ethylphenol,

vanillin/d_3-vanillin. Group 2: (*cis*-/*trans*-)whiskey lactone/d_2-(*cis*-/*trans*-)whiskey lactone, syringol/d_3-syringol, *p*-vinylguaiacol/d_3-*p*-vinylguaiacol. Group 3: 3-methyl-1-butanol/d_2-3-methyl-1-butanol, 2-methyl-1-propanol/d_2-2-methyl-1-propanol, 2-phenylethanol/$^{13}C_2$-2-phenylethanol. Group 4: syringaldehyde/d_2-syringaldehyde, eugenol/d_3-eugenol, β-damascenone/d_4-β-damascenone, ethyl cinnamate/d_5-ethyl cinnamate, β-ionone/d_3-β-ionone. Group 5: ethyl vanillate/d_5-ethyl vanillate, ethyl propanoate/d_5-ethyl isobutyrate, ethyl isovalerate/d_2-ethyl isovalerate, ethyl isobutyrate/d_5-ethyl isobutyrate. Group 6: isoamyl acetate/d_2-isoamyl acetate, phenethyl acetate/$^{13}C_2$-phenethyl acetate, ethyl butyrate/d_7-ethyl butyrate, ethyl hexanoate/d_{11}-ethyl hexanoate, acetic acid/d_3-acetic acid, butyric acid/d_7-butyric acid, isovaleric acid/d_2-isovaleric acid, phenylacetic acid/$^{13}C_2$-phenlyacetic acid, γ-nonalactone/d_2-γ-nonalactone.

For each group, seven solutions were created: 1 mL of solvent with 2 µL of each labeled standard solution, 1 mL of solvent with 2 µL of each unlabeled standard solution, 1 mL of solvent with 2 uL of labeled standard solution and 20 uL of unlabeled standard solution, 1 mL of solvent with 2 µL of labeled standard solution and 10 µL unlabeled standard solution, 1 mL of solvent with 2 µL of labeled standard solution and 2 µL of unlabeled standard solution, 1 mL of solvent with 10 µL of labeled standard solution and 2 µL of unlabeled standard solution, 1 mL of solvent with 20 µL of labeled standard solution and 2 µL of unlabeled standard solution. This was a total of 2 solutions for mass spectra and 5 solutions for calibration with mass ratios (unlabeled:labeled) of approximately 10:1, 5:1, 1:1, 1:5, and 1:10. Each solution was analyzed by GC-MS using cool, on-column injection, using an RTX-Wax column with checks on an SAC-5 column to confirm the accuracy of the calibration.

For each compound, the area of the selected mass ion on the chromatogram was integrated with the assistance of Enhanced Data Analysis Software (Agilent Technologies, USA). Using Microsoft Excel 2007, the actual mass ratio of the unlabeled/labeled compound was plotted against the

chromatogram area of the unlabeled/labeled compound. The slope of the line thus plotted was used to calculate the response factor for each compound: , where *m* is the slope of the line (Appendix B).

Quantification by Stable Isotope Dilution Analysis (SIDA)

Two whiskeys were analyzed by SIDA: Wild Turkey and Rittenhouse. Wild Turkey was selected because it had been the basis for both AEDA and SDA in order to determine the compounds further selected for quantification. Rittenhouse was selected in order to observe differences between rye whiskeys produced by different distillers. Wild Turkey was analyzed in triplicate samples from the same bottle to determine variability inherent in the method (from, for example, spiking error or integration error), while Rittenhouse was analyzed in duplicate. The difference in the number of replications was solely due to time and equipment constraints.

Five groups of target compounds were determined based on predicted concentrations and retention times (table 4.1). One group (E) was for high-concentration compounds suitable for analysis by cold-split (1:10) analysis for improved chromatography. Two groups (A, B) were selected as low-concentration or co-eluting compound requiring Selected Ion Monitoring (SIM) mode GC-MS analysis for accurate quantification. The other two groups (C, D) were compounds at sufficient concentration to quantify using Scan Mode analysis for accurate quantification.

For groups requiring SIM analysis, retention times (RTs) were obtained using authentic standards (table 4.1). SIM analysis used time-windows as broad as possible around each expected RT in order to fully capture both target compound and labeled analogue peaks. When possible, two ions for the target compound and two ions for the labeled analogue were monitored in SIM, in order to confirm the target peak.

For all compounds, integration was automatic when possible and manual when necessary, using the Enhanced Data Analysis Software package. Ratios of the integrated area for labeled and unlabeled

selected mass ions were calculated and used to determine the actual mass of the target compound as follows:

$$mass_{target} = R_f * mass_{labeled} * \frac{area_{target}}{area_{labeled}}$$

Determination of Non-Volatile Constituents

A single rye whiskey (Wild Turkey) was assessed for dissolved non-volatile compounds. In triplicate, 50 mL of rye whiskey was measured into a glass petri dish. The dishes were left uncovered but shielded from debris in a fume hood overnight (approximately 24 hours). The dishes were then exposed in a 115 ℃ oven for 2 hours, cooled in a desiccator, and the weight of each dish was recorded. The dishes were then returned to the same oven for an hour, cooled, weighed, and the process was repeated until no further weight change was recorded. The final mass of solids in the petri dish was considered the total mass of non-volatile constituents in 50 mL of rye whiskey.

III. RESULTS

Syntheses

Whiskey Lactone: The synthesis of d_2-whiskey lactone had an actual yield of 0.105 g, or 33.2% of total theoretical yield. Step 1, the synthesis of 2-methyl-1-hepten-3-ol, had a yield of 2.17 g, or 73.7% of theoretical yield. Step 2, the synthesis of 2-methyl-1-hepten-3-yl acryloylate, had a yield of 1.00 g, or 50.5% of theoretical yield. Step 3, the synthesis of dehydro-whiskey lactone, had a yield of 0.157 g, or 50.9% of theoretical yield. Because of the low yield of step 3, only 0.114 g (7.40×10^{-4} mol) of dehydro-whiskey lactone was used in Step 4. The theoretical yield of Step 4, therefore, was 0.117 g of d_2-whiskey lactone, and so the yield for Step 4 was 89.7% of theoretical yield.

Ethyl Esters: For the synthesis of d_5-ethyl cinnamate, yield was 0.1455 g, or 97% of theoretical yield. The yield of d_5-ethyl vanillate was 0.0941 g, or 52.9% of theoretical yield. The yield of d_5-ethyl isobutyrate was 0.0251 g, or 26.9% of theoretical yield. The actual yield of d_2-ethyl isovalerate was 0.0397 g, or 26.4% of theoretical yield.

Acetate Esters: The yield of d_2-isoamyl acetate was 0.0989 g, or 56.9% of theoretical yield. The yield of $^{13}C_2$-phenylethyl acetate was 0.0136 g, or 51.2% of theoretical yield.

Calibration of Stable Isotopes

(Appendix 3: Calibration Curves). Of the 31 compounds calibrated in the described fashion, all had response factors between 0.25 and 1.7. $^{13}C_2$-2-phenylethanol had the lowest response factor, with $R_f = 0.267$, and butyric acid had the highest, with $R_f = 1.649$. The calibration checks against the SAC-5 column did not reveal any significant differences in response factors between the two columns.

Quantification by SIDA

All 31 compounds targeted for quantification were subjected to SIDA. Abundance varied tremendously in the first whiskey analyzed (table 4.1), Wild Turkey, with ethyl cinnamate being the least abundant compound (7 µg/L) and 3-methyl-1-butanol being most abundant (2560000 µg/L). Standard deviations normalized by means (that is, standard deviation as a percentage of the actual mean value) ranged from 2% to 52%, although the latter was an extreme outlier, for β-damascenone, presumably explicable by the extremely low abundance of that compound (48 µg/L). Standard deviation generally increased dramatically with less abundant compounds, like the norisoprenoids and ethyl cinnamate, and with esters, which were difficult to quantify because of some interference from ethanol. In general, the median and mean percent standard deviations clustered around 10% (mean = 11%, median = 9.5%).

The second whiskey analyzed by SIDA, Rittenhouse (table 4.1), showed some variability in concentrations of individual compounds from Wild Turkey, as well as a generally lower abundance of most compounds. The variability of each compound's concentration seemed less (standard deviation % mean = 8.2%, median = 5%), but this may in part be explicable by duplications, rather than triplications. In particular, 4-ethylphenol and 4-ethyl-2-methoxyphenol were much less abundant in Rittenhouse than Wild Turkey. The ordering of compounds according to concentration also varied somewhat between the two whiskeys. This is to be expected from slight differences in production protocols.

Determination of Non-Volatile Constituents

After 4 hours of baking at 100 ℃ the petri dishes no longer showed any change in weight. The final mean weight of non-volatile constituents in 50 mL of whiskey was determined to be 74.1 mg, with a standard deviation of 1.6 mg. Thus, the concentration of non-volatile constituents in Wild Turkey rye whiskey is 1.48 g/L, with a standard deviation of 32 mg/L.

IV. DISCUSSION

Synthesis of Stable Isotopes

While Poisson and Schieberle provide a synthesis scheme for whiskey lactone that may have been less arduous (4), their method deuterates the linear carbon chain of whiskey lactone, rather than the lactonic ring itself. This method is appropriate for Chemical Ionization (CI) Mass Spectrometry, because it is a relatively "gentle" method of ionization which results in very little fragmentation. The present study, however, utilized Electron Ionization (EI) Spectrometry, which causes more extensive fragmentation. The largest fragment of whiskey lactone is the lactonic ring, with an undeuterated mass-to-charge ratio (m/z) of 99 (figure 4.1). It is thus necessary to deuterate this ring, which the synthesis scheme accomplishes by synthesizing an unsaturated lactone, which, when deuterated and analyzed by EI spectrometry results in a large base peak at m/z 101 (figure 4.1).

The synthesis scheme used was adapted from a published synthesis for massaiolactone, which is a six-membered pyranone, rather than a five-membered furanone (72). As a result, it is not surprising that Step 3 had a comparatively poor yield. The bond strain to form a five-membered ring using Grubb's Catalyst was probably more severe than it was for forming a six-membered ring in the original synthesis. Furthermore, the Grubb's Catalyst formed a floculant in dichloromethane – it was therefore necessary to remove the dichloromethane and reconstitute the reaction mixture in diethyl ether, and to remove the Grubb's Catalyst using a Florisil column, a step which undoubtedly contributed to the loss of product.

The two alcohol esters which were identified as important by dilution analysis were isoamyl acetate and phenylethyl acetate. Under EI spectrometry, the acetate fragment is entirely lost, making it necessary to isotopically label the precursor alcohol. In the case of 2-phenylethanol (the alcohol precursor to phenylethyl acetate) it is necessary to use a carbon-13 label, rather than a deuterium label, because the hydrogen exchange from the aromatic exchange makes a stable deuterium isotope unfeasible. The low yields of both alcohol esters are probably attributable to loss in both solvent removal under nitrogen purge and loss in the washing step of the synthesis, as both esters are somewhat water soluble.

The label location for the acid esters was determined by the presence of a detectable mass ion in their EI spectrum. In the case of ethyl cinnamate, ethyl vanillate, and ethyl isobutyrate, the mass ion peak was large enough for quantitation, and so it was possible to label them using deuterated ethanol (d_5-EtOH). In the case of ethyl isovalerate, there was no detectable mass ion peak in the EI spectrum, and so d_2-isovaleric acid was esterified with unlabeled ethanol. The low yield for many of these esters is again attributable to their moderate solubility in water and high volatility, making the wash and subsequent concentration steps significant factors for loss of the end product.

Quantification of Important Odorants in American Rye Whiskey

In general, while calculated concentrations in Wild Turkey rye are similar in order of magnitude to those reported in bourbon by Poisson and Schieberle (4) (table 4.1, figure 2.10), they are in every case pair-wise higher. Rittenhouse rye, on the other hand, has calculated concentrations much closer to those reported in that study. Unfortunately, although the same number of odorants were analyzed in the current research and the Poisson and Schieberle study, only 18 of the compounds are common to both, making a side-by-side comparison more difficult.

It is interesting to note that OAVs for Wild Turkey (table 4.1) reinforce the conclusions drawn from the dilution analyses in Chapter 3 (table 3.1). For example, ethyl propanoate – the only target

compound to be found below its reported threshold in either whiskey – was identified only in AEDA, not in SDA. Since AEDA involves introducing a potential extraction bias towards some compounds, it is reasonable to think that this is further evidence of this potential bias: ethyl propanoate could be considered to have been overly enriched during extraction, and so was erroneously identified as a potent odorant in AEDA, while in SDA it was not identified as an important odorant.

In general there is a very good correspondence between compounds with high OAVs and high FDs – further indication that direct injection, both in dilution analysis and in quantification, can provide excellent results with reduced sample preparation and bias introduction.

Of the important odorants in American rye whiskey, an overwhelming number likely originate from wood pyrrolysis. Yeast-fermentation compounds – the fusel alcohols – were indisputably important, but it is clear that lignin-derived compounds – phenols, guaiacols, and vanillin – and lactones, especially whiskey lactone, are the most important compounds, measured both by FD (from chapter 3) and, in this chapter, by OAV and abundance.

V. FIGURES AND TABLES

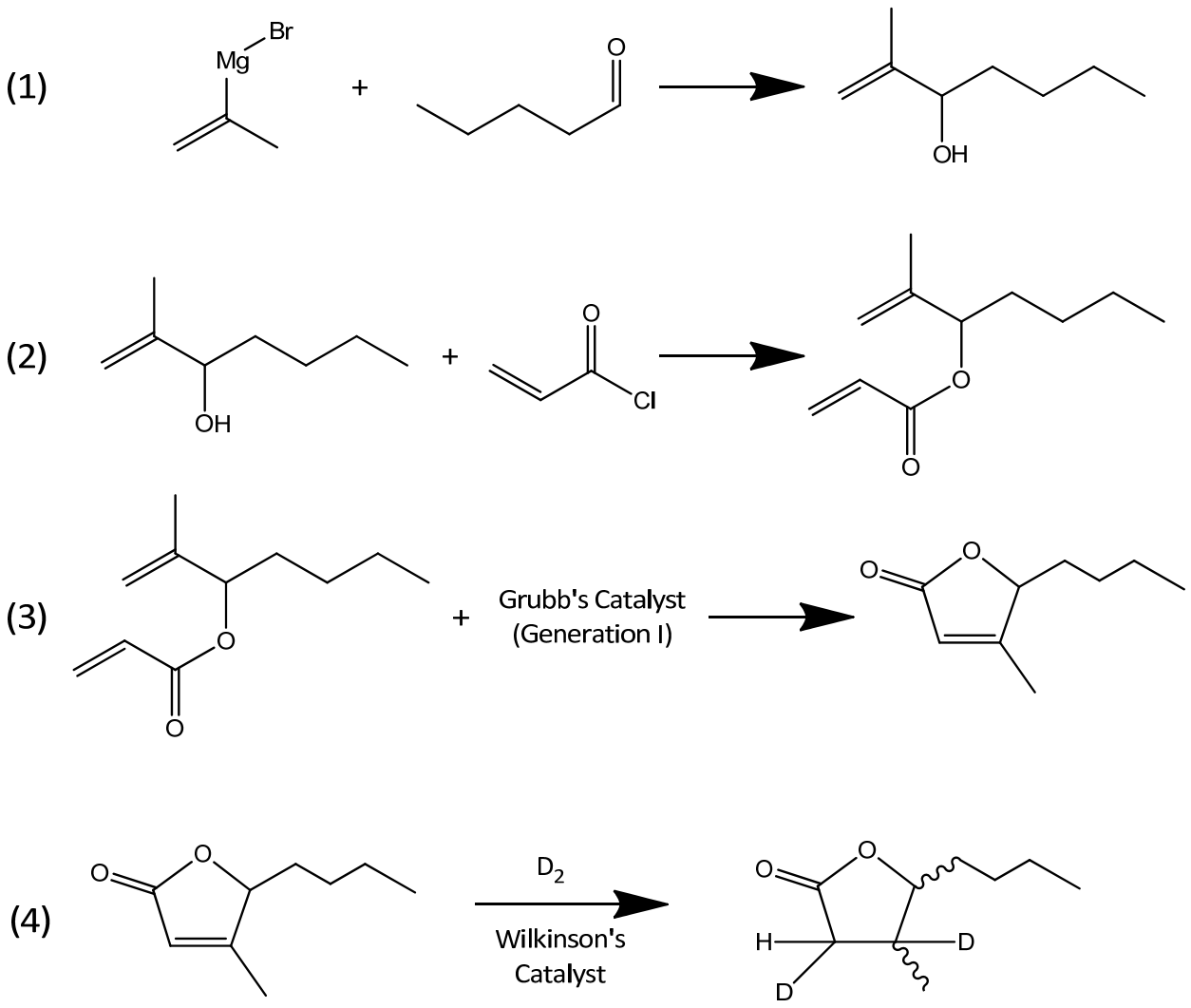


Figure 4.1: Synthesis scheme for d_2-whiskey lactone.

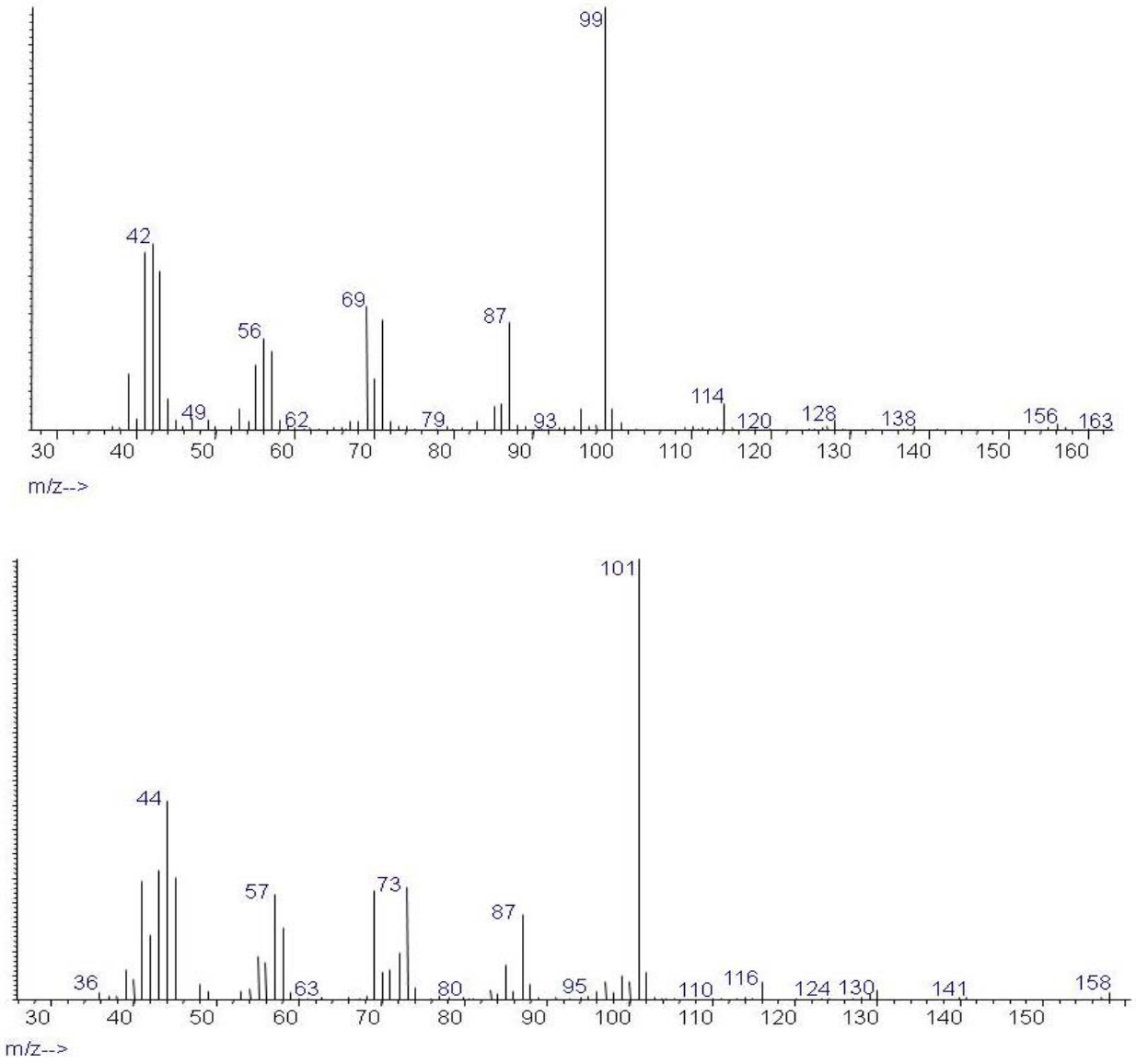


Figure 4.2: Electron Ionization (EI) spectrum of *cis*-whiskey lactone (top) and d_2-*cis*-whiskey lactone (bottom), demonstrating the main base peak at mass-to-charge 99/101.

compound	odor threshold (μg/L) in water/ethanol (6:4 by vol.)	OAV[a]
ethanol	[b]	12690
(*S*)-ethyl 2-methylbutanoate	0.2	138
3-methylbutanal	2.8	122
4-hydroxy-3-methoxybenzaldehyde	22	97
(*E*)-damascenone	0.1	79
ethyl hexanoate	30	67
ethyl butanoate	9.5	58
ethyl octanoate	147	57
2-methylpropanal	5.9	39
(3*S*,4*S*)-*cis*-whiskylactone	67[c]	37
(*E*,*E*)-2,4-decadienal	1.1	35
4-allyl-2-methoxyphenol	7.1	34
ethyl 3-methylbutanoate	1.6	33
ethyl 2-methylpropanoate	4.5	30
1,1-diethoxyethane	719	21
3-methyl-butanol	56100	19
2-phenylethyl acetate	108	18
(*E*)-2-nonenal	0.6	16
2,3-butandione	2.8	12
3-methylbutyl acetate	245	11
4-ethyl-2-methoxyphenol	6.9	9
γ-nonalactone	21	6
2-methoxyphenol	9.2	6
2-phenylethanol	2600	5
(*E*,*Z*)-2,6-nonadienal	0.3	3
trans-ethyl cinnamate	0.7	2
ethyl propanoate	3452	<1
(3*S*,4*R*)-*trans*-whiskylactone	790[c]	<1
(*E*,*E*)-2,4-nonadienal	2.6	<1
(*E*)-2-decenal	5.2	<1
γ-decalactone	21	<1

Figure 4.3: Orthonasal thresholds of selected compounds in a 60:40 water:ethanol matrix. Listed OAVs are from Poisson's and Schieberle's 2008 study of aroma compounds in American bourbon whiskey (4).

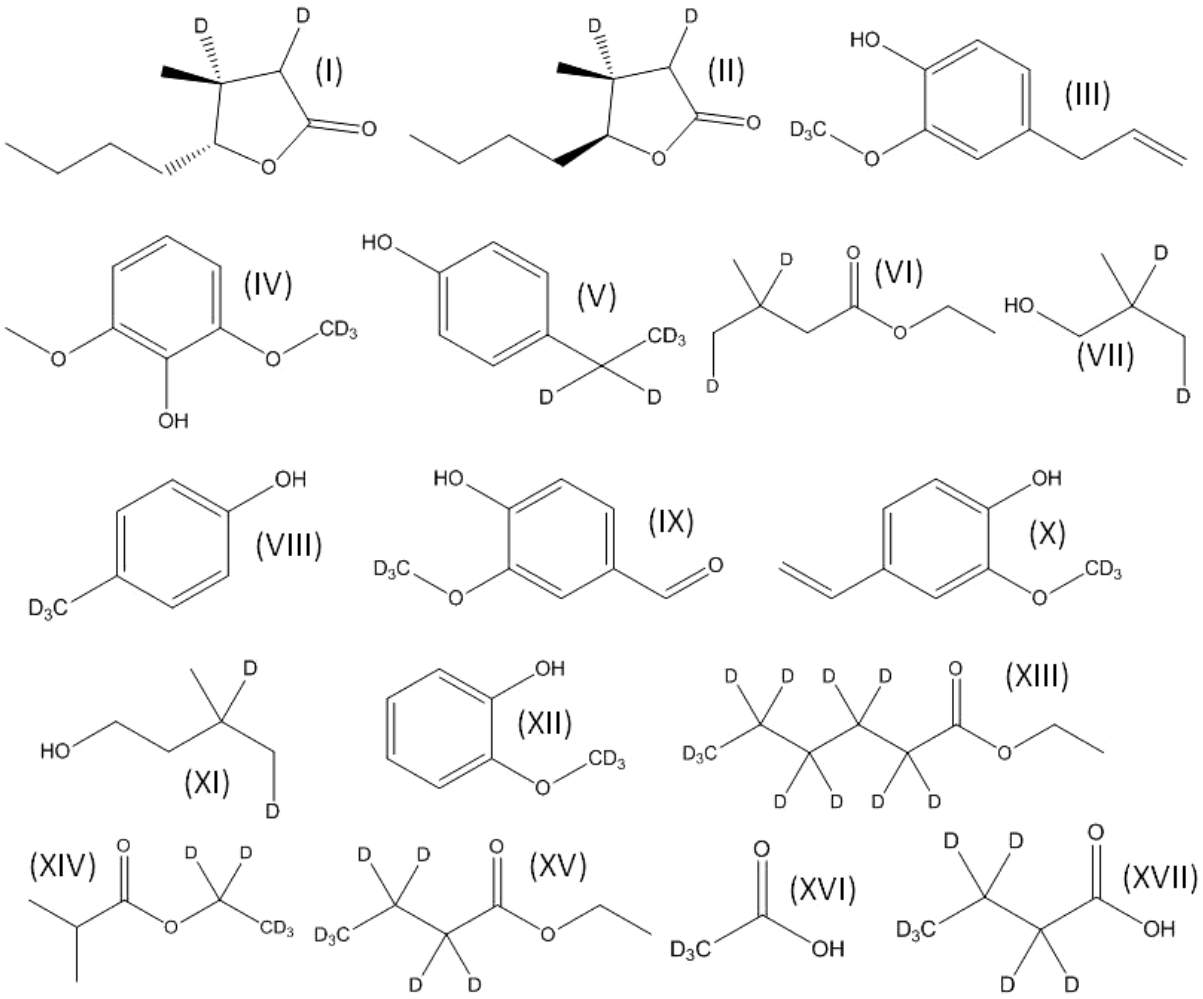


Figure 4.4: Molecular structures of deuterated standards, indicating the location of isotopic labeling. Standards are as follows: (I) d_2-*trans*-whiskey lactone, (II) d_2-*cis*-whiskey lactone, (III) d_3-eugenol, (IV) d_3-syringol, (V) d_5-4-ethylphenol, (VI) d_2-ethyl isovalerate, (VII) d_2-2-methyl-1-propanol, (VIII) d_3-*p*-cresol, (IX) d_3-vanillin, (X) d_3-*p*-vinylguaiacol, (XI) d_2-3-methyl-1-butanol, (XII) d_3-guaiacol, (XIII) d_{11}-ethyl hexanoate, (XIV) d_5-ethyl isobutyrate, (XV) d_7-ethyl butyrate, (XVI) d_3-acetic acid, (XVII) d_7-butyric acid.

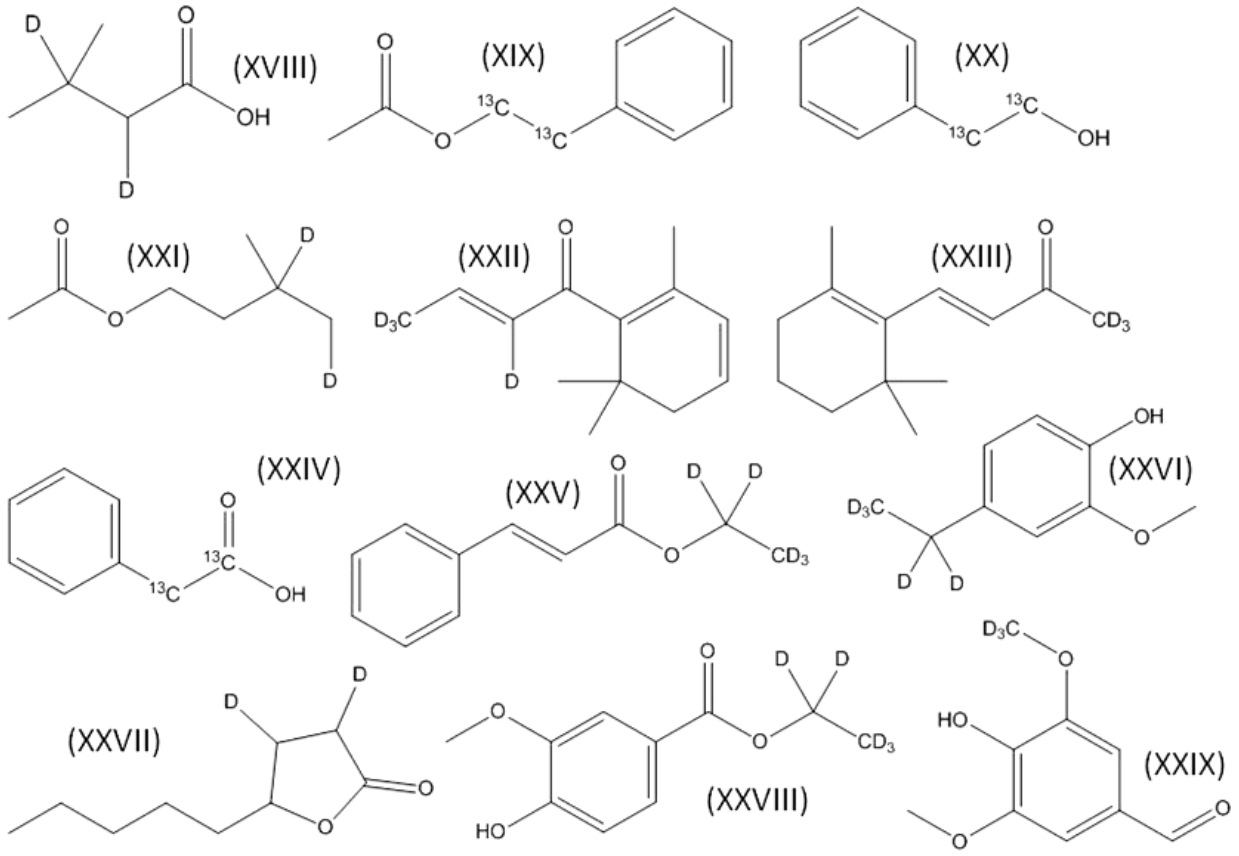


Figure 4.4 (continued): Standards are as follows: (XVIII) d_2-isovaleric acid, (XIX) $^{13}C_2$-phenylethyl acetate, (XX) $^{13}C_2$-phenylethanol, (XXI) d_2-isoamyl acetate, (XXII) d_4-β-damascenone, (XXIII) d_3-β-ionone, (XXIV) $^{13}C_2$-phenylacetic acid, (XXV) d_5-ethyl cinnamate, (XXVI) d_5-4-ethyl-2-methoxyphenol, (XXVII) d_2-γ-nonalactone, (XXVIII) d_5-ethyl vanillate, (XXIX) d_3-syringaldehyde.

Table 4.1: Concentration of Important Odorants in Two American Rye Whiskeys

Compound	Wild Turkey[a] Concentration (µg/L)	Wild Turkey[a] Odor Activity Value[c]	Wild Turkey[a] Standard Deviation (%)[a]	Rittenhouse[b] Concentration (µg/L)	Rittenhouse[b] Odor Activity Value[c]	Rittenhouse[b] Standard Deviation (%)[b]
ethyl cinnamate	7	10	14	5	7	20
β-ionone	32	[c]	22	31	[c]	10
p-cresol	41	[c]	15	42	[c]	1
β-damascenone	48	480	52	36	360	3
ethyl isovalerate	292	183	7	201	126	19
ethyl vanillate	520	[c]	5	340	[c]	1
eugenol	583	82	6	993	140	8
γ-nonalactone	684	33	3	231	11	5
4-ethylphenol	801	[c]	6	85	[c]	21
p-vinylguaiacol	848	[c]	6	608	[c]	7
ethyl isobutyrate	1020	[c]	11	734	[c]	2
trans-whiskey lactone	1300	2	13	1020	1	3
ethyl propanoate	1300	<1	17	1340	<1	13
ethyl butyrate	1680	177	17	1860	196	23
ethyl hexanoate	2180	73	3	1340	45	4
4-ethyl-2-methoxy-phenol	2180	316	9	187	27	22
phenylethyl acetate	2700	26	21	1910	18	10
guaiacol	3760	409	13	3150	342	1
2,6-dimethoxyphenol	5360	[c]	16	4910	[c]	4
isovaleric acid	5520	[c]	7	4090	[c]	4
phenylacetic acid	8030	[c]	4	12000	[c]	18
vanillin	8130	370	7	5610	255	11
cis-whiskey lactone	8370	125	3	5300	79	9
isoamyl acetate	8760	36	10	7270	29	1
butyric acid	9570	[c]	10	8430	[c]	9
syringaldehyde	13800	[c]	5	9550	[c]	5
2-phenylethanol	20100	8	4	5180	2	4
acetic acid	805000	[c]	12	539000	[c]	4
2-methyl-1-propanol	1150000	[c]	11	496000	[c]	4
3-methyl-1-butanol	2560000	46	2	1620000	29	2

[a]Wild Turkey samples were run in triplicate from the same bottle. Mean standard deviation was 11%, while median standard deviation was 9.5%. [b]Rittenhouse samples were run in duplicate from the same bottle. Mean standard deviation was 8.2%, while median standard deviation was 5%. [c]Odor Activity Value (OAV) is calculated as actual concentration of an odorant divided by its odor threshold in the matrix. Threshold values are from Poisson and Schieberle (4) and are based on a 60:40 water:ethanol matrix; some compounds do not have a reported threshold in this matrix (figure 3.3).

--5. Sensory Studies on Whiskey Model Solutions--

I. JUSTIFICATION OF RESEARCH AND OBJECTIVES

The use of GCO and quantitative techniques to identify important odor-active compounds in food products is an essential step in understanding the contribution of these compounds to the ultimate sensory perception of a food product. However, all of these *in vitro* methods do not correspond to the everyday experience of a consumer, expert or inexperienced, during the consumption of the product. In order to determine whether the results of the analytical methods – the compounds identified and quantities calculated – accurately capture the important organoleptic qualities of a food product, it is necessary to conduct sensory assays with human subjects based on the data gathered in the analytical phase of the research.

In the previous chapters, the techniques for first identifying – by Gas Chromatography-Olfactometry (GCO) and dilution analyses – and then quantifying – by Stable Isotope Dilution Analysis (SIDA) – important odorants in American rye whiskey were employed to provide a list of these important odorants (table 3.1) and quantification of these odorants (table 4.1). In the research detailed in the current chapter, these data are used to construct a model whiskey and to determine whether this model whiskey is confusable with the authentic whiskey on which it is based.

Previous research in whiskey model verification (4) employed a descriptive analysis panel in order to determine the confusability of the model with the whiskey on which it was based (figure 5.1). Unfortunately, descriptive analysis is not meant to determine the confusability or discriminability of two products; it is meant to provide a sensory-conceptual mapping of a group of related but non-confusable products. In the case of the Poisson and Schieberle study (4), the employment of descriptive analysis without first determining whether the products were confusable means that it is possible that an attribute not accounted for in descriptive analysis would lead to significant discriminability between the

two samples – while the spider-web diagrams appear to indicate confusability, they are not statistically significant.

The present research, therefore, was aimed at determining whether the model constructed using the analytical techniques detailed in previous chapters would be confusable with the authentic sample. Rather than use the commonly employed difference tests (duo-trio, triangle, etc), this study employed the R-Index by Rating method, for several reasons. First, using the R-Index Method is generally easier for judges and provides better results (74).

Second, the R-Index method is more powerful than other difference tests: if judges are used as instruments and not meant to represent the general population, only a small number of judges are necessary (74). Third, R-Index allows for inferential comparison of multiple samples without exhaustive pair-wise sampling; that is, it is possible to compare many samples with a fraction of tests that such an experimental design would require from a triangle or duo-trio test. Finally, the R-Index by Rating is a very simple task for panelists (75), requiring a simple pairwise comparison between two samples. This task requires minimal training and does not exhaust a judge unduly. It is thus possible to gather significant information using a relatively small number of judges and tests.

II. MATERIALS AND METHODS

Materials

Four different commercial rye whiskeys, which are widely available nationally, were purchased at a local liquor store (Savoy, IL). Three of the four whiskeys – Jim Beam Rye, Old Overholt, and Rittenhouse – had a reported ethanol content by volume of 40%; the fourth whiskey – Wild Turkey Rye – had a reported ethanol v/v of 50.5%. Each was declared to be a straight rye whiskey according to government specifications (2). Mention of the brand names of these whiskeys does not imply any research contact or sponsorship, and is not for advertisement or endorsement purposes.

All authentic standards except for β-damascenone were of ≥98% purity; β-damascenone had a measured purity of 95.01%. Authentic standards were obtained from the following sources. Acros (New Jersey): ethanol. Sigma-Aldrich (St Louis, MO): ethyl butyrate, ethyl caproate (hexanoate), ethyl propanoate, butyric acid, ethyl isovalerate, phenylacetic acid, ethyl isobutyrate, phenethyl acetate, 4-ethylphenol, p-cresol, γ-nonalactone, eugenol, 2,6-dimethoxyphenol, isovaleric acid, syringaldehyde, cis+trans-whiskey lactone (52.2% trans), β-ionone, guaiacol, 2-phenylethanol. Alfa Aesar (Lancaster, UK): ethyl cinnamate, 4-ethyl-2-methoxyphenol, ethyl vanillate. Avocado (Lancaster, UK): 4-vinyl-2-methoxyphenol. Baker (Phillipsburg, NJ): 2-methyl-1-propanol. Firmenich (Switzerland): β-damascenone. Fisher (Fair Lawn, NJ): acetic acid, sulfuric acid, methanol, diethyl ether, pentane, 3-methyl-1-butanol, acetaldehyde. Fluka (Switzerland): vanillin, isoamyl acetate.

Determination of Acetaldehyde in American Rye Whiskey

Acetaldehyde in whiskey was determined using an external calibration method. Pure acetaldehyde (obtained as described below) was used to create solutions of known concentration in 50.5% ethanol/water (v/v): 25 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 1000 ppm. Each solution was analyzed by GC-FID (parameters described below), and the area of the peak was determined. Peak area was plotted against concentration using Microsoft Excel 2007, and the linear slope of the regression was used as the response factor of the FID to acetaldehyde. Authentic rye whiskey was then analyzed using the same GC-FID method, and the peak area of acetaldehyde was plotted using the calculated response factor to determine its concentration in whiskey.

GC-FID Parameters: GC-FID was conducted using an Agilent 6890 Gas Chromatograph (Agilent, Santa Clara, CA), equipped with a Flame Ionization Detector. A Restek RTX-Wax column (Restek, Bellefonte, PA) of 15 m length, 0.53 mm internal diameter, and 1 μm internal film was used. A Gerstel CIS-4 Programmable Temperature Vaporizer (PTV) inlet (Gerstel, Mülheim an der Ruhr, Germany) was used for all injections.

Injection volume of all samples was 1 µL, and the inlet was operated in splitless mode throughout, with a purge at 1.00 minutes. Total runtime was 44.5 minutes. The flow was directly transferred through deactivated, uncoated fused silica tubing to a Flame Ionization Detector (FID) operating at 250 ℃. The CIS-4 inlet was programmed to hold at -120 ℃ until injection and for 0.10 minutes afterward. After that point, the initial inlet ramp was 10 ℃/s for 10 minutes, to a final temperature of 260 ℃. The oven was set to an initial temperature of 20 ℃ using cryonic cooling, held for 5 minutes, after which it was ramped at a rate of 10 ℃/min to a hold temperature of 200 ℃.

Purification of Acetaldehyde

Acetaldehyde is both extremely volatile, boiling well below room temperature. It also trimerizes into paraldehyde even at low temperatures during storage. It was therefore necessary to purify the standard. This was done by distillation (77) at room temperature. Approximately 10 mL of crude acetaldehyde was measured into a pre-chilled flask, acidified using a single drop of sulfuric acid in order to facilitate reaction of the paraldehyde trimer into acetaldehyde (77), and attached to a small distillation receiver, which was cooled to ∼-10 ℃ using a salt/ice slurry. The distillation flask was gently heated using warm water (ramping from 35 – 45 ℃) until approximately 2 mL of distilled acetaldehyde was collected. The distilled acetaldehyde was transferred to a chilled flask. A pre-weighed flask of 10 mL ethanol/water (50.5% v/v) was sealed using a PTFE septum, and the distilled aldehyde was transferred into this solution using a syringe. The change in weight of the flask was used to calculate the concentration of acetaldehyde in solution. The flask was left sealed, and stored at -70 ℃ until needed.

Isolation and Deodorization of Rye Whiskey Matrix

Wild Turkey rye whiskey (750 mL) was subjected to SAFE (78). The residue from the distillation flask was dissolved in 200 mL of methanol and 25 mL of water, and then allowed to evaporate overnight. The resulting slurry was subjected to HVT using a diffusion pump (at a vacuum of approximately 10^{-5} torr). The residue was dissolved in 15 mL ethanol/water (50.5% v/v), and once again subjected to HVT.

The flask and residue were weighed, and then the residue was dissolved in 30 mL of ethanol/water (50.5% v/v) and pipetted out. The empty flask was weighed and the difference was calculated to yield the final concentration of matrix. Aliquots of the matrix solution were diluted in ethanol/water (50.5% v/v) according to the pre-determined concentration (chapter 4) in whiskey, and transferred to PTFE sniff-bottles to determine whether any odor-activity was present.

Construction of Rye Whiskey Models

Rye whiskey models were constructed according to the absolute concentration data determined in chapter 4 (table 4.1). Authentic standard solutions were made up at 1000X concentration (that is, if desired final concentration was 10 ppm, a 10,000 ppm solution was made up). These solutions were then added to a volumetric flask, combined, and diluted 1000X, to yield the final product. The final model solution was stored at room temperature.

Correcting the Model Solution Against Authentic Whiskey

Aliquots (10 mL) of the initial model solution and authentic Wild Turkey rye whiskey were spiked with 100 µL of 99% 6-undecanone, a compound which does not occur naturally in whiskey and which does not co-elute on RTX-Wax columns with any of the previously identified important odorants. Model solution and authentic whiskey were both run in triplicate on a GCO/FID (parameters below). Of each triplicate set, one run of model solution and one of whiskey were monitored by sniffing to determine if the model differed detectably in any particular respect from the authentic whiskey.

Peak areas for each compound (determined by previous RT identification – chapter 3, table 3.1) were recorded for both the model and the authentic whiskey. Peak areas for 6-undecanone were recorded and the ratio of 6-undecanone between the authentic whiskey and model was used to correct for injection area. Because FID analysis does not allow for the possibility of de-convoluting co-eluting peaks, only peaks known to elute by themselves were corrected. Corrected concentrations for the model solution were calculated according to the following equation:

$$[compound]_{corrected} = [compound]/(\frac{area(compound)_{model}}{area(compound)_{whiskey}} * \frac{area(6-undecanone)_{whiskey}}{area(6-undecanone)_{model}})$$

Discriminability of Commercial Rye Whiskeys by the R-Index Method

Four American rye whiskeys – Rittenhouse (RH), Wild Turkey (WT), Old Overholt (OO), and Jim Beam (JB) – were diluted to 20% ethanol v/v using deodorized water, as is standard industry practice (60). Aliquots of diluted whiskey (20 mL each) were measured into clean, 125 mL Teflon bottles with vented caps for olfactory assessment (sniffing). Twenty bottles were filled, with 5 replications of each whiskey. Four sets of whiskeys were separated, each with a separate noise sample (that is, there was a set with RH as the noise, a set with WT as the noise, etc). Thus, each set had five sample bottles – a noise ("N") sample and four whiskeys, one of each type (including the noise), labeled with random, 3-digit numbers.

A panel of 17 people participated in this study: 5 males, 12 females, with ages ranging from 21 to 37. Possible color variations were obscured by wrapping the bottles in aluminum foil. Panelists were asked first to familiarize themselves with the noise sample, and then to determine whether each numbered sample differed from the noise (was a signal) and whether they were certain or not (the respective rating are SS, S?, N?, NS). Detailed instructions and release forms can be found in Appendix C.

R-Index values were calculated according to the following response matrix rating method:

	SS	**S?**	**N?**	**NS**
Sample	a	b	c	d
Noise	e	f	g	h

$$R = \frac{a(f+g+h) + b(g+h) + ch + \frac{1}{2}(ae + bf + cg + dh)}{n_S n_N}$$

where *a*, *b*, etc are the number of times each signal rating is assigned to each sample, and n_S and n_N are the number of sample and noise repetitions, respectively. An R-Index value within a range defined as

0.50 plus or minus a critical value (which varies based on Type I error tolerance and number of judges) is considered statistical proof on lack of discriminability. For each number of subjects and desired α, there is a corresponding threshold R-Index value, above which a sample is said to be significantly discriminable from the noise (79).

Discriminability of Whiskey Models and Commercial Whiskeys by the R-Index Method

The model whiskey was compared to all 4 commercial whiskeys using the R-Index by Rating method. Five sets (one for each commercial whiskey and the single model whiskey) of six 125 mL Teflon bottles were filled with 20 mL of whiskey or whiskey model solution, diluted to 20% ethanol v/v as above, for a total of 30 bottles. Each set had, as the noise sample, a different commercial whiskey or the model whiskey. Seven males and 6 females participated in this particular research, with an age range between 21 and 60 years. Noise samples were labeled "N", all other samples were assigned random 3-digit codes. The R-Index value for each whiskey was calculated as above.

III. RESULTS

Determination and Purification of Acetaldehyde

The peak area of acetaldehyde by GC-FID was plotted against concentration (in ppm) using Microsoft Excel (figure 5.2). The relationship was highly linear, with $R^2 = 0.9997$, and a response factor of 353829. All 4 whiskeys were analyzed for acetaldehyde content, and showed remarkably similar concentrations of acetaldehyde. The 95% confidence interval for acetaldehyde concentration in all whiskeys tested was between 31 and 49 ppm, with a mean value of 40 ppm. For individual whiskeys, the results were as follows:

Wild Turkey: mean = 37 ppm, 95% confidence from 35 to 39 ppm

Rittenhouse: mean = 41 ppm, 95% confidence from 27 to 55 ppm

Jim Beam: mean = 44 ppm, 95% confidence from 38 to 49 ppm

Old Overholt: 39 ppm, 95% confidence from 35 to 43 ppm

Acetaldehyde was successfully distilled from the crude acetaldehyde, with a yield of 225 mg of acetaldehyde. This was immediately stored in 50.5% v/v ethanol/water at -70 °C, in order to prevent formation of the acetal (1,1-diethoxyethane) by reaction with ethanol or the paraldehyde trimer by reaction with itself.

Isolation and Determination of Whiskey Matrix

A total of 1.371 g of dried material was recovered from the isolation process. This was dissolved in 30 mL of 50.5% v/v ethanol/water, to yield a concentrate of 45.7 mg/mL. This was stored at room temperature. When diluted to the previously calculated level of non-volatile material in whiskey (1.5 mg/mL) in 50.5% v/v ethanol/water, the deodorized matrix was not distinguishable from the pure ethanol/water mix. It was therefore considered sufficiently deodorized.

Construction and Correction of Model Whiskey Models

Two model whiskeys were constructed – one based directly on quantification performed in the previous chapter (chapter 4) – model A – and one based on corrected values calculated by internal standard comparison between model A and authentic Wild Turkey rye whiskey by GC-FID – model B (table 5.1). It was immediately apparent that model A was not a suitable model for comparison by difference tests with authentic rye whiskey: the two were not confusable. While descriptive analysis was not conducted during this research, sensory descriptors applied to model A included *medicinal*, *barnyard*, and *banana*, possibly indicating inappropriate concentrations of phenols, methoxyphenols, and isoamyl acetate. *Whiskey* or *whiskey-like* aroma descriptors were not forthcoming.

Corrected concentrations for odorants in model B were calculated by calibration with a separate internal standard, 6-undecanone. Ratios of peak area in model A and authentic Wild Turkey were further corrected using the ratio of 6-undecanone in each sample to correct for injection area. In model B, roughly half (17 compounds) were corrected, and acetaldehyde was added to the model. Many corrections were relatively minor (table 5.1), but some were quite dramatic. For example, 2-

phenylethanol was more than quadrupled in concentration, while isoamyl acetate was decreased by a factor of 3.

Acetaldehyde, as expected, made a large difference in the impression of the models, adding a pungency otherwise lacking.

Discriminability of Commercial Rye Whiskeys by the R-Index Method

Judges were not able to distinguish with any statistical significance between the 4 brands of commercial whiskey (table 5.2a-d). The only comparison that approached a significant difference was that of Old Overholt to Rittenhouse, with a R-Index = 0.70. Since most other R-Index scores were in the neighborhood of 0.5-0.6, it is hard to explain this high value. Olfactory fatigue is unlikely, both because samples were appropriately diluted according to accepted industry technique Judges were encouraged to assess both samples and sets in random order (verbally, by test coordinator).

Discriminability of Whiskey Models and Commercial Whiskeys by the R-Index Method

Once again, all of the commercial whiskeys were not well discriminated from each other (tables 5.2a-d, 5.3a-d). However, with Wild Turkey as the "noise" sample, the model whiskey was barely non-discriminable, with an R-Index value of 0.73 and a critical R-Index value of 0.74 (table 5.2a). Furthermore, when the model was used as the "noise" sample, *no* whiskey had an R-Index score higher than the critical value, although most were quite close (between 0.67 and 0.71).

IV. DISCUSSION

Early model mixtures constructed from quantification data were clearly not confusable with actual whiskey – descriptors used were *smoky*, *medicinal*, and *cough medicine*. In order to remedy this problem, two actions were taken. First, acetaldehyde, a potent odorant common to many food products, especially fermented foods and alcoholic beverages (76), was considered as a source of variation between the authentic product and the model. Acetaldehyde is difficult to quantify for a number of reasons: it is extremely volatile, with a boiling point of 21 ℃, and extremely labile, trimerizing

to form paraldehyde – a heterocyclic oxane – spontaneously. It is thus understandable that previous studies of whiskey have not attempted to investigate its role in the characteristic aroma of whiskey. This research was able to determine a GC-FID method, using cryonic cooling of both the injection inlet and the GC oven, to identify and quantify acetaldehyde in whiskey.

Second, because of the unique properties of whiskey (and other distilled alcoholic beverages) that make it suitable for direct analysis by GC methods (see chapters 2 and 3), it was possible to directly compare the model whiskey to the authentic product by GC-FID in order to investigate their comparative compositions; that is, it was possible, through the use of careful internal standardization and injection techniques, to compare actual quantities of odorants in the model whiskey, *post-formulation*, to those odorants in actual whiskey, and to adjust levels in the model accordingly. This opportunity is usually not available, as direct comparison in-GC of products is complicated by the impossibility of direct injection of model and authentic product.

According to Poisson and Schieberle (4), a deodorized whiskey matrix improved confusability between their model whiskey and the authentic product (figure 5.1). The concentration of rye whiskey matrix, defined as the non-volatile, odorless, dissolved constituents of whiskey, was determined in the previous chapter (chapter 4). SAFE and high-vacuum transfer (HVT) were used to isolate the matrix from the volatile constituents of whiskey (including the ethanol and water that make up 99.9% of the whiskey's volume). The final product was dissolved in an appropriate (50.5% ethanol v/v) ethanolic matrix and used in model solutions.

Before the final model whiskey was compared to authentic whiskeys in a difference test, the four commercial whiskeys used throughout this research (Wild Turkey, Jim Beam, Old Overholt, Rittenhouse) were compared through the R-Index by Rating method. This served two purposes: first, to determine whether the assumption that Wild Turkey was an appropriate representative whiskey for further, detailed analysis (including the model construction being tested) held true in sensory testing.

Second, this test determined whether it would be possible to compare the model whiskey against *all* of the whiskeys in this research, or, if they were significantly different from each other, if the model could only be tested against authentic Wild Turkey. Because the test showed that all whiskeys were highly confusable, despite their differing chemical compositions (table 4.1), it was possible to compare the model whiskey against all of the commercial whiskeys.

Acetaldehyde is known to be an important contributor to the flavor of many foods and beverages – it is known to be present in whiskey in significant amounts well over its reported threshold in water (76, 80). It gives a characteristic *pungent, sharp, fermented* note to foods. With reported values of up to 150 ppm (76), acetaldehyde is one of the more abundant trace compounds reported in whiskey. The concentrations reported in this research – around 50 ppm – fall within the normal range of acetaldehyde abundance in whiskey. It is interesting to note the remarkably similar concentrations of acetaldehyde in all 4 commercial whiskeys thus surveyed – the means for all 4 whiskeys clustered right around the group mean of 40 ppm. This is especially interesting given that Wild Turkey has an ethanol percentage by volume higher by 10.5% than the other whiskeys – more variation in acetaldehyde would have seemed normal from this difference.

Similarly, it is interesting to note the inability of judges to discriminate between different brands of American rye whiskey, despite presumably different initial mash bills, distilling processes, and aging protocols. There are several possible interpretations for this failure of discrimination. First, it is possible that American rye whiskey, as a product, has a strong, unique aroma profile that supersedes brand any differences introduced by individual distillers. Second, it may be that these 4 products are, in fact, very similar in production process, and so coincidentally have similar aroma profiles *despite* originating from different distillers. It would be illuminating to introduce into the product mix several whiskeys that are definitively different – a bourbon, for example, in order to illuminate sources of variation that come

from mash bill differences, or several ryes from the same distiller but aged for different lengths of time, in order to explore the effect of longer exposure to the charred oak on the flavor of whiskey.

Finally, it is possible that untrained judges are poor discriminators of whiskey. As demonstrated by this research, American rye whiskey is a complex mix of potent odorants at concentrations well above their aroma threshold. It is possible that an expert panel would be more able to distinguish between whiskeys with more exactitude – in the commercial and industrial world, there is certainly a higher value placed on the opinion of expert judges for discriminating whiskeys than is placed on the opinion of untrained judges (1, 60). Training a panel (as is standard for descriptive analysis) would probably lead to different results than those of a naïve panel. It would be illuminating to compare R-Index results for the discrimination of different commercial whiskeys before, during, and after such training.

The results of the model study – the R-Index test with the model and commercial whiskeys – showed that the model whiskey, while not perfect, is substantially correct. Before discussing the model study, it is worth noting that, when technically confusable, the R-Index values for the model whiskey hovered very close to the border of discriminability. Furthermore, the relatively low number of judges adds to the possibility of one or two very poor discriminators skewing the results of the test. However, as it is possible to conduct an R-Index study with as few as 5 judges (79), these somewhat positive results are at the very least encouraging for further research.

The model whiskey was based on the quantification data from Wild Turkey American rye whiskey. It is therefore encouraging that, of the 4 commercial whiskeys it was compared to, Wild Turkey was the one with which it was most confusable. Unfortunately, this research did not construct models based on the other whiskeys, and so it is impossible to definitively rule out a testing error in order to account for this happy coincidence.

It is also worth noting that the model was confusable with all of the whiskeys when the model was the "noise" sample. Unfortunately, this may detract from the validity of the model study, rather than bolster it: since the model was not confusable with any whiskey besides Wild Turkey during the other tests (table 5.3b-d), the fact that it is suddenly confusable in one test points more towards some kind of testing error than to an ideal model. Nevertheless, once again this is cause for at least cautious optimism about the accuracy of the model (and thus the quantitation behind its construction).

Finally, it is worth once again mentioning the experience – or lack thereof – with whiskey that the judges possessed. Judges were not screened for interest in or expertise with whiskey or any other alcoholic beverage. It is highly possible that, had this information been collected, judges would have separated into distinct groups based on experience – those with more expertise with whiskey being better able to distinguish the model sample, while those without experience discriminating poorly. From anecdotal debriefings after testing, it was clear that those with more whiskey expertise were better able to distinguish the model sample from authentic whiskeys.

The relative success of this model study is worth comparing to the model study of bourbon whiskey published by Poisson and Schieberle (4). In that study, the authors used descriptive analysis to evaluate the success of their model. They evaluated their model against authentic bourbon based on eight attributes – *malty*, *fatty*, *coconut-like*, *fruity*, *flowery*, *vanilla-like*, *smoky*, and *phenolic* – using chemical standards to train panelists and a rating system to construct spider-web studies (figure 5.1). Unfortunately, the authors did not perform any statistical analysis of these data; they concluded that their model was similar based on similar ratings to authentic whiskey on these eight attributes. It is worth remembering that descriptive analysis is not meant to judge confusability; descriptive analysis is a technique meant to further *describe* the differences between samples known to be non-confusable.

Therefore, the current research can be considered to be a fairly unique success. In no way is the model, and the quantification upon which it is based, believed to be perfect – in fact, the authors are

able to easily distinguish the model from whiskey in any rigorously blinded test, due to extreme familiarity with the authentic whiskeys and the model compounds. The model's confusability with authentic whiskey, however, indicates that the current work is substantially correct – it is sensory validation – of a kind – of the instrumental and analytical quantification of chapters 3 and 4. More work is necessary – there are many strategies that can be employed to create a model which better approximates – and, ultimately, imitates – authentic rye whiskey; these strategies will be briefly explored in the next chapter. However, as far as the current research is concerned, this sensory work is confirmation of accurate identification and quantification of the key aroma compounds of American rye whiskey.

V. FIGURES AND TABLES

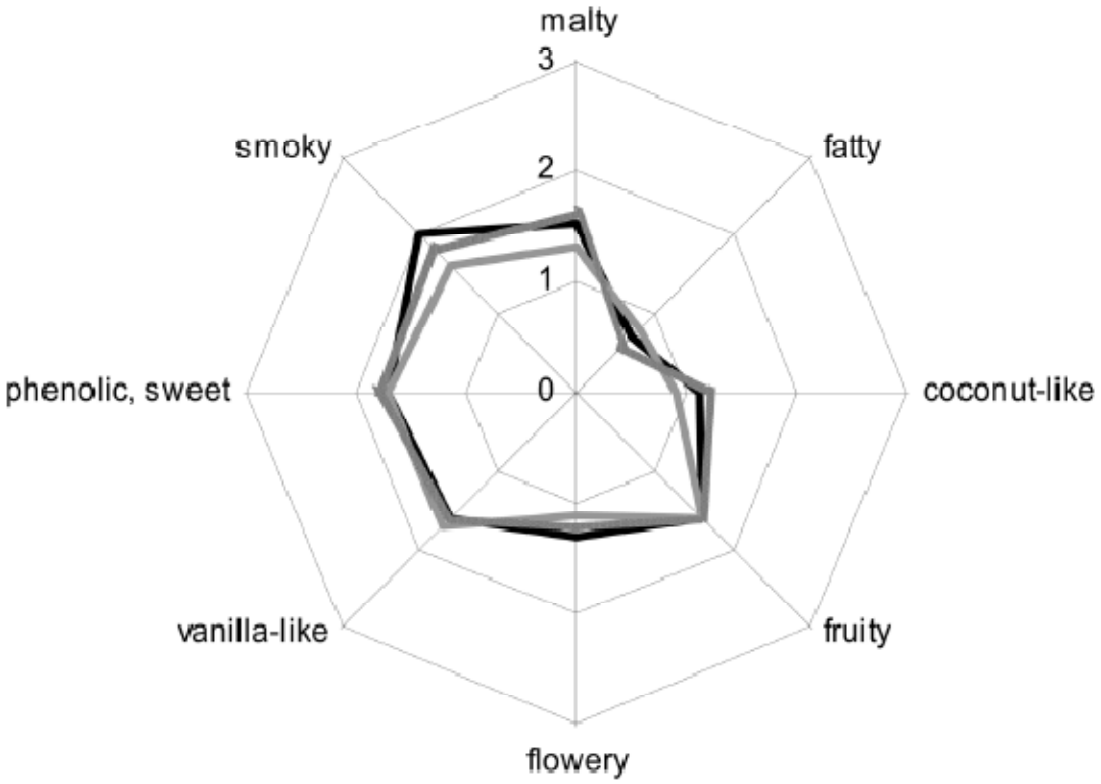


Figure 5.1: Descriptive analysis attribute diagram of American bourbon whiskey (black), model bourbon whiskey (grey) and model bourbon whiskey with nonvolatile whiskey matrix (dark grey). While the spider-web patterns appear similar, it is possible that a quality not listed here would account for significant variation between samples (4).

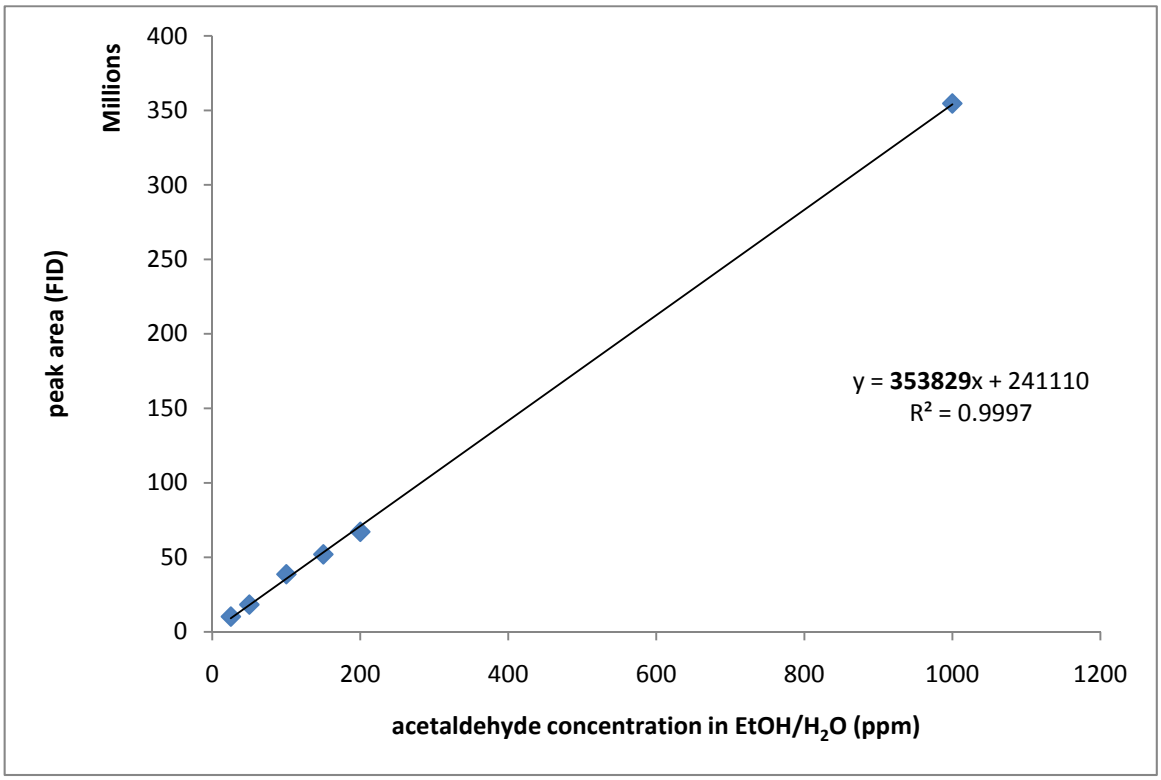


Figure 5.2: Peak area of acetaldehyde from GC-FID as a function of concentration (ppm). The response factor (in bold) can be used to calculate the concentration of acetaldehyde in a sample.

Table 5.1: Comparison of whiskey models A and B.

Compound	Model A ppb (μg/L)	Model B ppb (μg/L)
2,6-dimethoxyphenol	5400	3000
2-methyl-1-propanol	1100000	820000
2-phenylethanol	20000	92000
3-methyl-1-butanol	2600000	2800000
4-ethyl-2-methoxyphenol	2200	2200
4-ethylphenol	800	800
acetaldehyde	N/A	40000
acetic acid	800000	640000
butyric acid	9600	2900
cis-whiskey lactone	8300	8000
ethyl butyrate	1700	1700
ethyl cinnamate	7	7
ethyl hexanoate	2200	3600
ethyl isobutyrate	1000	1000
ethyl isovalerate	290	290
ethyl propanoate	1300	1300
ethyl vanillate	520	840
eugenol	580	890
guaiacol	3800	3800
isoamyl acetate	8800	3900
isovaleric acid	5500	3100
p-cresol	41	41
phenylacetic acid	8000	8000
phenylethyl acetate	2700	1700
p-vinylguaiacol	850	850
syringaldehyde	14000	62000
trans-whiskey lactone	1300	1300
vanillin	8100	8100
β-damascenone	48	48
β-ionone	32	32
γ-nonalactone	680	1500

Model A is based on quantification results from chapter 4 (table 4.1), while model B is based on a comparison of model A and authentic rye whiskey (Wild Turkey) by internally standardized GC-FID. Acetaldehyde was not quantified in chapter 4, and so was not included in model A. Percent change is as a percent of the concentration in model B.

Table 5.2a: Rittenhouse R-Index Scores (Rittenhouse as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Rittenhouse*	*3*	*4*	*8*	*2*	*0.50*
Old Overholt	6	9	0	2	0.70
Wild Turkey	2	4	6	5	0.42
Jim Beam	3	4	5	5	0.45

*Noise Sample

Table 5.2b: Old Overholt R-Index Scores (Old Overholt as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Rittenhouse	4	4	4	5	0.54
Old Overholt*	*2*	*5*	*5*	*5*	*0.50*
Wild Turkey	5	5	4	3	0.62
Jim Beam	1	8	2	6	0.51

*Noise Sample

Table 5.2c: Wild Turkey R-Index Scores (Wild Turkey as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Rittenhouse	6	5	3	3	0.54
Old Overholt	5	2	5	5	0.43
Wild Turkey*	*5*	*5*	*4*	*3*	*0.50*
Jim Beam	6	3	5	3	0.50

*Noise Sample

Table 5.2d: Jim Beam R-Index Scores (Jim Beam as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Rittenhouse	5	4	5	3	0.53
Old Overholt	6	5	3	3	0.57
Wild Turkey	6	2	5	4	0.52
Jim Beam*	*5*	*4*	*3*	*5*	*0.50*

*Noise Sample

Critical R-Index value is 0.72 for $n = 17$ and a two-tailed $\propto = 0.05$ (79). Thus, none of the 4 rye whiskeys sampled in this research is significantly distinguishable from any other.

Table 5.3a: Model vs Commercial Whiskeys (Wild Turkey as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Wild Turkey*	*4*	*1*	*6*	*2*	*0.5*
Rittenhouse	0	4	5	4	0.37
Old Overholt	4	2	7	0	0.57
Jim Beam	4	3	5	1	0.57
Model	11	0	0	2	0.73

*Noise Sample

Table 5.3b: Model vs Commercial Whiskeys (Rittenhouse as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Wild Turkey	2	5	3	3	0.53
Rittenhouse*	*3*	*2*	*5*	*3*	*0.5*
Old Overholt	4	3	3	3	0.56
Jim Beam	2	3	3	5	0.44
Model	11	0	0	2	0.77

*Noise Sample

Table 5.3c: Model vs Commercial Whiskeys (Old Overholt as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Wild Turkey	5	1	3	4	0.61
Rittenhouse	1	5	5	2	0.63
Old Overholt*	*1*	*2*	*6*	*4*	*0.5*
Jim Beam	5	2	4	2	0.69
Model	11	0	0	2	0.84

*Noise Sample

Table 5.3d: Model vs Commercial Whiskeys (Jim Beam as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Wild Turkey	3	7		3	0.62
Rittenhouse	4	1	5	3	0.53
Old Overholt	3	0	6	4	0.45
Jim Beam*	*3*	*1*	*6*	*3*	*0.5*
Model	11	0	0	2	0.77

*Noise Sample

Table 5.3e: Model vs Commercial Whiskeys (Model as "Noise")

Whiskey	SS	S?	N?	NS	R-Index Value
Wild Turkey	9	3	0	1	0.71
Rittenhouse	7	3	2	1	0.67
Old Overholt	8	3	1	1	0.69
Jim Beam	10	0	1	2	0.7
Model*	*5*	*1*	*0*	*7*	*0.5*

*Noise Sample

Critical R-Index is 0.74 for $n = 17$ and a two-tailed $\alpha = 0.05$ (79). The model whiskey is well-discriminated from all whiskeys except for Wild Turkey (5.3a), but all whiskeys are confusable with the model whiskey (5.3e).

--6. Conclusions--

The goal of the current research was to identify and quantify the key odorants in American rye whiskey – a distilled alcoholic beverage of historical, cultural and economic importance – using a non-extractive technique optimized for this sort of highly ethanolic beverage, meant to provide a sensitive, accurate, and quick alternative to more common solvent extractions or volatile-adsorption methods. In addition, basic sensory evaluation of a model constructed from the available data was conducted in order to determine the accuracy and efficacy of the aroma analysis and novel methodology.

A total of 30 key odorants were identified using a combination of the new technique – Sample Dilution Analysis – and the current gold standard technique – Aroma Extract Dilution Analysis. In addition, acetaldehyde was identified as an important contributor to rye whiskey's unique flavor. All of these compounds were successfully quantified, and a model whiskey was constructed which performed adequately, but not perfectly, in difference testing against authentic whiskeys.

Future research should focus on sensory-guided aroma identification in order to better understand the deficiencies of the current model. To a judge familiar with whiskey, there are clear areas of difference between the model and the authentic whiskey, but these areas are not well understood. By approaching the problem from a sensory science perspective it may be possible to more effectively focus on specific compounds and odorants than by painstakingly evaluating every chemical identifiable by analytic means.

For example, a descriptive analysis panel would *now* be the appropriate next step for this research. Work by Lee *et al* (35) has already established a flavor wheel for whiskeys – this can be used to guide an exploration of the differences between the current model and the authentic product. Once an area of deficiency is well-identified and understood (the lack of a *woody* note, for example), analytical research could be focused on identifying compounds present in authentic whiskey which might be responsible for such an impression.

Another aspect of whiskey that was not well-explored by the current research is the top note of whiskey – the extremely volatile fraction poorly evaluated by extractive techniques, and often difficult to evaluate even by GC methods. Acetaldehyde is a good example of this type of compound – on most GC phases it elutes far too quickly to be identified, much less quantified. It is also difficult or impossible to extract, again due to its volatility. It is possible that whiskey has a number of other compounds of this type responsible for the initial, orthonasal impression, and work should be focused on identifying these extremely volatile odorants.

Finally, there are a number of volatile and non-volatile compounds in whiskey that are not odor-active, but may have a profound effect on aroma synergism, by affecting partitioning or sequestration of actual odorants (14, 52, 53, 81, 82). It is not just possible but almost certain that this type of effect – of long-chain ethyl esters and other congeners that are not aroma-active *per se* – is essential to creating authentic whiskey aroma.

The current research attempted to address this type of effect in a brief manner by creating a deodorized whiskey matrix using SAFE and HVT, but such a procedure is little more than a shot in the dark. Detailed study of aroma-*in*active volatile and non-volatile congeners in whiskey would surely lead to a greater understanding of the aroma system. If a true American rye whiskey model is to be attempted, these are areas of study which should be further explored. The current research, while hopefully useful, is a very small start to an incredibly broad and complex area of study.

--7. Appendix A: Detailed Syntheses—

Synthesis of $^2H_2$-Whiskey Lactone

Reagents:

- **Isopropenylmagnesium bromide solution**, 0.5 M in THF (Aldrich 419567-100ML)
- **Pentanal** (Valeraldehyde, 97%; Aldrich 110132-250-ML; F.W. 86.13)
- **Acryloyl chloride**, 98% (Aldrich 549797-5G; F.W. 90.51)
- **Et_3N** , triethylamine (Aldrich 471283-100ML; F.W. 101.19)
- **Ti(*i*-PrO)$_4$**, titanium isopropoxide (Aldrich 377996-5ML; F.W. = 284.26)
- **Grubbs Catalyst**, Generation I (Aldrich 579726-1G; F.W.= 822.96)
- **Et_2O**, diethyl ether
- **CH_2Cl_2**, dichloromethane
- Wilkinson's catalyst [Chlorotri(triphenylphospine)rhodium(I)] (Aldrich 199982-5G)
- UHP deuterium gas
- Methanol-d

Procedure:

Synthesis of 2-methyl-1-hepten-3-ol *(F.W. = 128)*

1) Set up a dry 200-mL round bottom flask with mechanical stirrer and N_2 purge. Maintain N_2 purge throughout the reaction.
2) Transfer 50 mL of the isopropenylmagnesium bromide solution (25 mmol) to the flask.
3) Cool the flask in an ice-water bath.
4) While stirring the above solution, add (slowly) 2.0 g of pentanal (23 mmol) dissolved in 5 mL of THF.
5) Continue stirring for 4-6 hours (at 0 °C). [Make sure the flask is sealed properly to minimize any chance of moisture getting into the reaction mixture.] Check progress of the reaction by GC-MS (by appearance of product and disappearance of pentanal). If the reaction is not complete by end of day store the reaction flask in the refrigerator and work-up the next morning.
6) [N_2 purge is not needed for the following steps.] The next morning, cool the flask in an ice-water bath, then add (dropwise) 50 mL of saturated NH_4Cl solution (aqueous) to the flask. Check pH of the aqueous phase to make sure it is neutral or slightly acidic.
7) After 15 minutes, add an additional 30 mL of dd H_2O.
8) Allow the solution to come to room temperature, remove the THF layer (keep it) and then extract aqueous phase with Et_2O (2 x 25 mL).
9) Dry the THF-Et_2O extract over anhydrous sodium sulfate.
10) Distill off most of the solvent using a Vigreaux setup.
11) Distill the product under high vacuum (HVT) to give 2-methyl-1-hepten-3-ol.
12) Remove the solvent and determine the yield (theoretical = 0.023 mol = 2.944 g).

Synthesis of 2-methyl-1-hepten-3-yl acryloylate *($C_{11}H_{17}O_2$; F.W. = 182)*

1) Set up a dry 100-mL round bottom flask with a mechanical stirrer and rubber septum (with nitrogen purge and vent needles). [Keep the reaction under nitrogen purge throughout the experiment.]

2) Dissolve 1.28 g of 2-methyl-1-hepten-3-ol (0.010 mol) and 1.21 g of Et_3N (0.012 mol) in 30 mL of CH_2Cl_2.
3) Cool the flask to 0 °C in an ice-water bath.
4) While stirring, slowly add 1.08 g of acryloyl chloride (0.012 mol dissolved in ~ 5 mL of CH_2Cl_2).
5) Continue stirring for 6 hours at 0 °C. *[Check for product formation by GC-MS. For the analysis remove about 100 uL of reaction mixture and add to a 2-mL vial containing 1 mL of water and 0.5 mL of CH_2Cl_2.]
6) Once the reaction is complete, at 0 °C (flask temperature), add 20 mL of dd H_2O and continue stirring until all the precipitate has been dissolved. Collect the CH_2Cl_2 layer.
7) Extract the mixture 3 x 30 mL with Et_2O.
8) Wash the Et_2O + CH_2Cl_2 extract (2 x 10 mL) with a 10% H_2SO_4 solution.
9) Wash the extract (2 x 10 mL) with aqueous saturated sodium bicarbonate solution.
10) Subject to HVT to recover the ester. Remove the solvent and determine the yield (theoretical = 0.01 = 1.82 g).

***Note:** If the reaction does not appear to go to completion add a few more drops of acryloyl chloride (the solution might turn yellow and smoke a bit during the addition). After 1 h, the reaction should be checked again by GC-MS.

Synthesis of dehydro-whiskey lactone *(F.W. =154)*

1) Set up a three-neck dry 100-mL round bottom flask with a mechanical stirrer, condenser (4 °C) and rubber septa (with nitrogen purge and vent needles). Maintain nitrogen purge throughout the experiment.
2) Add 0.364 g of *2-methyl-1-hepten--3-yl acryloylate* (0.002 mol) to the flask and dissolve in 50 mL of CH_2Cl_2.
3) Add 0.33 g of Grubbs Catalyst, Generation I (0.0004 mol = 20 mol % of 0.002 mol) and 300 μL of Ti(*i*-PrO)$_4$.
4) Reflux the mixture for 12 hours; periodically check product formation by GC.
5) Attach a Vigreaux column to the reaction flask. Evaporate the CH_2Cl_2 until the reaction mixture is a brown sludge. Redissolve mixture in 50 mL of Et_2O.
6) Pass the Et_2O extract through 15 g of Florisil to remove the Grubb's Catalyst.
7) Wash the Et_2O extract with saturated Na_2C0_3 (3 x 25 mL) and then saturated NaCl (3 x 25 mL).
8) Dry the Et_2O extract over anhydrous Na_2SO_4 and evaporate off the excess solvent to yield the product. (Theoretical yield = 0.002 mol = 0.308 g).

Synthesis of $^2H_2$-whiskey lactone *(F.W. = 158)*

1) Weight the dehydro-whiskey lactone (0.2 g) into a pressure reactor (equipped with a rubber septum) along with 30 mg (15% w/w) of Wilinson's catalyst.
2) Add 3 mL of MeOD (deuterated methanol), a stir bar and seal the reactor and connect the deuterium line (via a needle that is placed below the solution). While stirring, apply deuterium pressure and insert a vacuum line (via a needle) and vent the vial for 1 or 2 minutes to completely flush the reactor with deuterium.
3) Remove the vacuum line and pressurize the reactor to 20 psi.
4) Monitor the reaction via GC-MS by using a 10 uL syringe to remove the reactant/product mixture for analysis. The catalyst will usually turn from red/orange to light yellow/white when the reaction is completed.

5) When completed, carefully remove the deuterium line (do not turn off the deuterium flow before doing this or the solution will suck back into the line) from the reactor and then turn of the deuterium flow.
6) Work up the product by extracting with ether and centrifuging to remove the spent catalyst.
7) Remove the solvent and purify the product by flash chromatography using 10:90 ether/pentane as mobile phase. (Theoretical yield = 0.002 = 0.316 g).

References: (72, 83)

Grignard method: Vogel's Textbook of Practical Organic Chemistry, 5th edition. 1989. Furniss et al. (Eds.). Longman Group UK Ltd., p. 539.

Synthesis of Labeled Acetate Esters

Reagents

- **$^{13}C_2$-2-phenylethanol**
- **$^{2}H_2$-3-methyl-1-butanol**
- **acetyl chloride**
- **triethylamine**
- **CH_2Cl_2 (dichloromethane)**
- **Et_2O (diethyl ether)**
- **anhydrous Na_2SO_4**
- **10% H_2SO_4**
- **Saturated $NaHCO_3$**

Procedure:

1) Set up a dry 100-mL round bottom flask with stir bar and rubber septum. Insert N_2 purge and vent needles.
2) Cool flask to 0°C in an ice bath.
3) Dissolve labeled alcohol (0.5 g; 3.52 mmol) and triethylamine (4.224 mmol) in 20 mL of methylene chloride, add to flask.
4) Slowly add acetyl chloride (4.224 mmol) in 5mL methylene chloride to the stirring solution.
5) Purge with nitrogen gas for additional 10 minutes before removing needles and sealing the septum.
6) Leave stirring for 6 hours.
7) Quench reaction with 20 mL of water and let stir until precipitate has dissolved.
8) Collect the methylene chloride layer and re-extract with ether (2 x 15 mL).
9) Wash with 10% sulfuric acid then saturated sodium bicarbonate (2 x 10 mL)
10) Dry over anhydrous Na_2SO_4 and remove solvent.

Synthesis of Labeled Ethyl Esters

Reagents

- **$^{2}H_2$-isovaleric acid**
- ***trans*-cinnamic acid**

- **vanillic acid**
- **isobutyric acid**
- **$^2H_5$-ethanol**
- **H_2SO_4 (sulfuric acid)**
- **saturated Na_2CO_3**
- **anhydrous Na_2SO_4**
- **diethyl ether (Et_2O)**

Procedure:

1) Combine 1 mmol acid standard with 1 mmol of $^2H_5$-ethanol (or, in the case of $^2H_2$-isovaleric acid, with unlabeled ethanol) in a 4 mL glass vial with Teflon cap.
2) Add 1 drop of concentrated H_2SO_4 to the reaction mixture and seal the cap.
3) In a larger flask or beaker to protect against potential pressure buildup, incubate the reaction mixture for at least 12 hours, and overnight if necessary.
4) Neutralize the reaction mixture with 1 mL of saturated Na_2CO_3.
5) Extract reaction mixture (3x) with 2 mL Et_2O.
6) Backwash Et_2O extract (2x) with saturated Na_2CO_3.
7) Dry Et_2O extract with anhydrous Na_2SO_4.
8) Remove solvent under gentle N_2 to yield the labeled ethyl ester product.

Synthesis of $^2H_3$-2-Methoxyphenol (guaiacol), $^2H_3$-2-Methoxy-4-Methylphenol (4-methylguaiacol), or $^2H_3$-2-Methoxy-6-methoxyphenol (syringol)

Reagents

- **Catecol** (Aldrich 135011-100G; F.W. 110.11)
- **4-Methylcatecol** (Aldrich M34200-5G; F.W. 124.14)
- **4-Ethylcatecol**, 98%, 1 g (Alfa Aesar A12048/L02647; 138.17)
- **3-Methoxycatecol**, 98%, 5 g (Alfa Aesar B20006/L12876; F.W. 140.14)
- **3,4-Dihydroxy-5-methoxybenzaldehyde** (Aldrich 464120-1G; F.W. 168.15)
- **Iodomethane - d_3**, 99.5% atom %D (Isotech 176036-5G; F.W. 144.96)
- **Ethanolic (absolute) 2N NaOH** (4 g solid NaOH in 50 mL of absolute ethanol)
- **Aqueous 10% HCl**
- **Absolute ethanol**
- **H_2O**
- **CH_2Cl_2**

Procedure:

1) Place 1.10 g of catecol (10 mmol) OR 1.24 g of 4-methyl catecol (10 mmol) OR 1.40 g of 3-methoxycatecol (10 mmol) into a 50-mL screw cap test tube. [For 4-ethylcatecol the recipe will need to be halved, 0.69 g = 5 mmol].
2) Add 10 mL of ethanolic 2N NaOH + 10 mL of ethanol. [The additional ethanol is necessary to thin the solution sufficiently so it can be stirred.]
   - The catecol solution should turn an olive green/grey color.
   - The 4-methyl catecol solution should turn blue/green/black

3) Add 10 mL of ethanolic 2N NaOH + 10 mL of ethanol. [The additional ethanol is necessary to thin the solution sufficiently so it can be stirred.]
   - The catecol solution should turn an olive green color.
   - The 4-methyl catecol solution should turn a blue/blue-black color.

4) Over the course of < 2 minutes, add dropwise a 2 mL ethanolic solution of 1.45 g of CD_3I (10 mmol).
5) Seal the flask with PTFE-lined cap, shake vigorously (with periodic venting) then continue stirring for 48 h. [Checked 24 h product by taking about 250 uL of reaction mixture and diluting into about 1 mL of 2M HCl + 0.5 mL ether.]
6) Using 100 mL of H_2O solubilize and transfer the reaction product to a 250-mL separatory funnel.
7) Extract with ether (3 x 25 mL) to remove the dimethylated product.
8) Acidify (pH < 2) the aqueous phase using 50 mL of aqueous 4 M HCl (saturated with NaCl) and extract with ether (3 x 25 mL).
9) Reduce the ether extract volume to about 5-10 mL and subject to HVT.
10) Check purity by GC-MS (neat injection).

Reference: (66)

Synthesis of $^2H_3$-Eugenol

Reagents:

- **Eugenol**, 99% (Aldrich E5,179-1; F.W. 164.20)
- **Aluminum chloride**, 99% (Aldrich 237051-100G; F.W. 133.34)
- **N,N-Dimethylaniline**, 99% (Aldrich 515124-100ML; F.W. 121.18; d = 0.956)
- **Iodomethane - d_3**, 99.5% atom %D (Isotech 176036-5G; F.W. 144.96)
- **Toluene** (dry)
- **H_2O**
- **Ether**

Procedure:

Step 1: Synthesis of 4-Allylcatecol (2AC) (F.W. 150)[1]

1) Place 10 mL (9.6 g) of N,N-dimethylaniline (79 mmol) into a 100-mL round bottom flask equipped with a reflux condenser, stir bar and nitrogen purge line.
2) Add (slowly, with stirring) 10.6 g (80 mmol) of aluminum chloride and then add toluene (5 mL) – stir for 15 min. [The solution heats up and smokes during the addition – final solution is tea brown but clear.]
3) To the above mixture add (drop by drop) 6.56 g of eugenol (40 mmol) dissolved in 15 mL of toluene. [The solution gets hot and starts to boil during the addition.]
4) Reflux the mixture to 100-110 °C for 2 h (actual temp = 108 °C). [The solution gets thick and some gelatinous material forms, but the solution is stir stirable.]
5) Cool the mixture and then slowly drop into ice-cold water (50 mL) with stirring. [Solution starts out clumpy and thick and then thins with stirring. Color is brownish/grey.]
6) Adjust pH to 1-2 and transfer the mixture to a separatory funnel. Recover toluene layer and then extract 3X with ether (20 mL). [Toluene does not seem to extract the product – stays clear with a green thick layer underneath on top of the aqueous layer (sandwiched).]

7) Backwash the extract (toluene+ether) with aqueous 2N HCl (2 x 20 mL).
8) Backwash the extract against aqueous 2N NaOH (3 x 15 mL) – recover NaOH layer which is brown in color.
9) Adjust pH of aqueous extract to 1-2 and then extract with ether (3 x 25 mL). [Ether layer extracts the tea brown colored product.]
10) Dry the extract (about 75 mL) over 10 g of anhydrous sodium sulfate. Purge with nitrogen and store in freezer until next step.
11) Remove ether to yield the crude 4-allycatecol.
12) Check yield by mass recovered after flash chromatography (1:1 P/E). Yield = 5.32 g. Use product for next step.

Step 2: Synthesis of $^2H_3$-2-methoxy-4-allyl-phenol ($^2H_3$-eugenol)^2

1) Place 1.5 g of 2AC (10 mmol) into a 50-mL screw cap test tube (equipped with PTFE lined cap).
2) Add a stir bar and 10 mL of ethanolic 2N NaOH. [A clear deep reddish brown solution formed.]
3) Under a gentle stream of nitrogen add dropwise a 10 mL ethanolic solution of 1.45 g of **iodomethane - d_3** (10 mmol).
4) Purge the tube with N_2 and seal with a PTFE-lined cap. Shake vigorously (with periodic venting) then continue stirring for 72 h. On occasion, invert the tube and continue stirring to ensure all areas in the tube react sufficiently.
5) Pour the reaction mixture into a 500-mL beaker and evaporate off the ethanol using a combination of warming (external water bath) and N_2 purging.
6) Using 150 mL of H_2O, solubilize and transfer the reaction product to a 250-mL separatory funnel.
7) Extract with CH_2Cl_2 (4 x 20 mL) to remove the dimethoxylated product.
8) Adjust pH to <1 and extract with CH_2Cl_2 (3 x 20) to isolate the methoxyphenol product
9) Backwash the ether extract with aqueous 1 N NaOH solution (3 x 15 mL) to isolate the methoxyphenol product.
10) Adjust the pH of the NaOH extract to <1 using 4N HCl and extract with ether (3 x 15 mL).
11) Wash the ether extract with water (2 x 10 mL) and then dry over anhydrous sodium sulfate.
12) Reduce the ether extract volume to about 5-10 mL and subject to HVT.
13) Subject to flash chromatography on silica gel using 80:20 pentane/CH_2Cl_2 as mobile phase.
14) Check yield and purity by GC-MS (yield = 0.1748 g).

Reference: (66, 71)

Synthesis of $^2H_3$-syringaldehyde

Reagents:

- **3,4-dihydroxy-5-methoxybenzaldehyde** (Aldrich 464120-1G; F.W. 168.15)
- **dimethylsulfate-d_6** (Aldrich 16,452-6; F.W. 132.18; density = 1.397)
- **aqueous 40% (w/v) potassium hydroxide solution**
- **aqueous 4N HCl solution**
- **H_2O**
- **ethyl acetate**

Procedure:

1) Place 0.50 g of 3,4-DH5MB (3 mmol) in a 50-mL round bottom test tube (with PTFE-lined cap) equipped with N_2-purge and stir bar.
2) Add 5 mL of aqueous 40% KOH.
3) Over the course of 30 minutes (5-6 drops every 5 minutes) add 0.42 g of d_6-DMS (3.2 mmol). [The reaction mixture starts out yellowish brown and slightly cloudy.]
4) Cap the vial and stir for 2 h. [Solution turns dark brown/green and viscous/cloudy.]
5) Chec reaction by GC-MS. For this, take 5 drops of reaction solution and add to a vial containing 1 mL aqueous 1N HCl and 0.5 mL ethyl acetate. Mix thoroughly and analyze the ethyl acetate layer.
6) Add more d_6-DMS as necessary until nearly all starting material has been consumed.
7) Once the reaction is complete, acidify the aqueous phase using 4N HCl and extract with ethyl acetate (1x10 mL, 4x5 mL).
8) Wash extract with saturated NaCl solution (3x10 mL)
9) Dry with anhydrous Na_2SO_4
10) Filter solution through glass wool and evaporate solvent with N_2-purge and hot water bath. [Note that syringaldehyde is not very volatile, and so will not evaporate readily, making solvent removal easier.]
11) Weigh final product and resolubilize in diethyl ether for storage.

Reference:

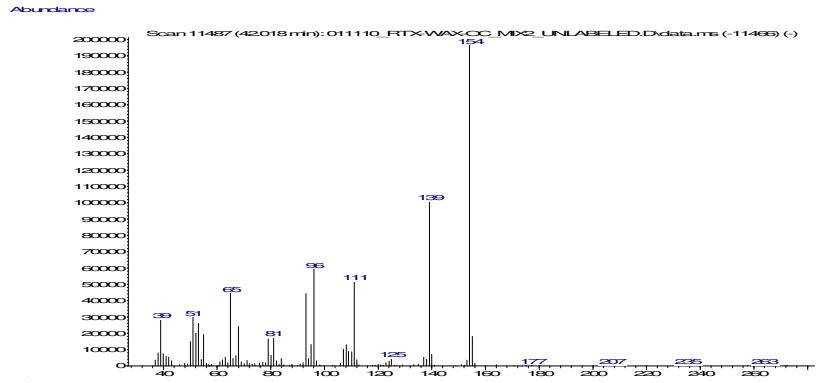
Experiment 6.111 "Cognate preparations: *Varatraldehyde*" in *Vogel's Textbook of Practical Organic Chemistry*, 5th edition. 1989. Furniss *et al* (Eds.). Longman Group UK Ltd, p. 987.

--8. Appendix B: Calibration Curves--

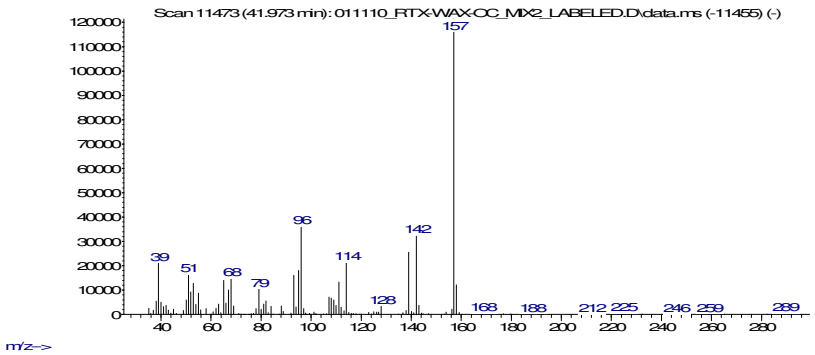
Response Factor of d_3-syringol (January 13, 2010)

	Isotope	Unlabelled
Standard:	2-[$^2H_3$]-methoxy-6-methoxyphenol	2,6-dimethoxyphenol
CAS:	NA	91-10-1
Mfg/Reference:	NA	Sigma-Aldrich (St. Louis, MO)
No.; Catalog #; Batch#/Lot#:	NA	611; D135550-25G; 06013TD
% Purity (by GC-FID)	99.9%	99.9%

Spectra



Unlabeled

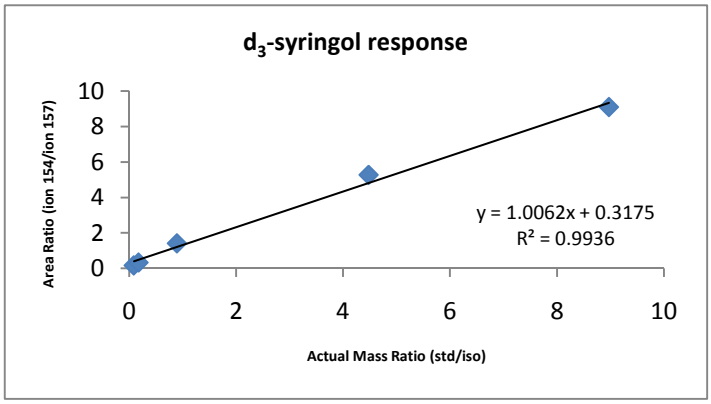


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		157	154
Mass Ratio:	0.0897	39548321	6743093
	0.179	16362967	5386712
	0.897	4347852	6139936
	4.48	5012495	26457010
	8.97	5793842	52740377

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

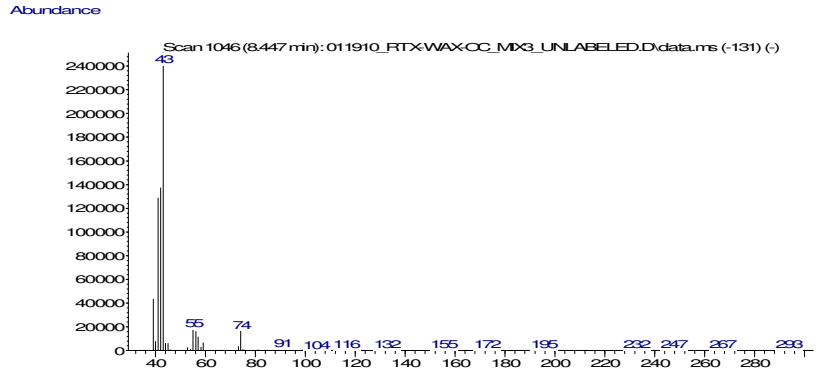


slope =	1.0062
response factor =	**0.994**

Response Factor of d_2-2-methyl-1-propanol (February 10, 2010)

	Isotope	Unlabelled
Standard:	2-methyl-[2,3-$^2H_2$]-1-propanol	2-methyl-1-propanol
CAS:	NA	123-51-3
Mfg/Reference:	NA	Baker, Phillipsburg NJ
No.; Catalog #; Batch#/Lot#:	NA	188
% Purity (by GC-FID)	99.75%	99.9%

Spectra



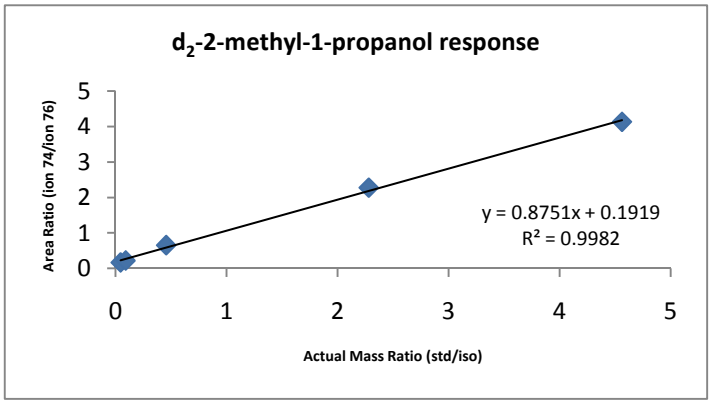
Unlabeled

Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		76	74
Mass Ratio:	0.0456	9503562	1619473
	0.0912	8335451	1882920
	0.456	1116861	734789
	2.28	1094336	2492773
	4.56	1234569	5101083

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

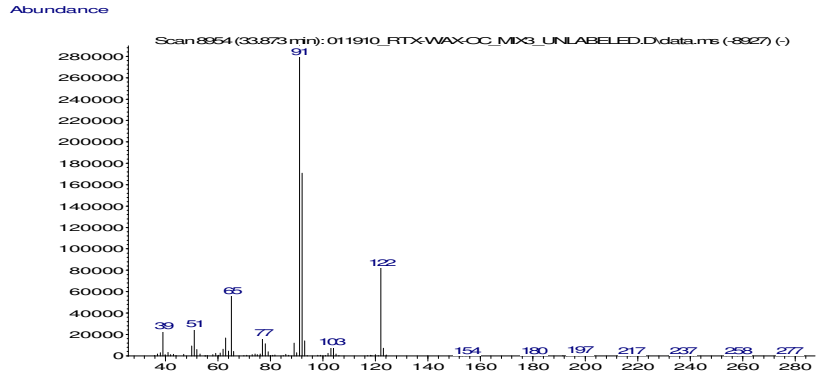


slope =	0.8751
response factor =	**1.14**

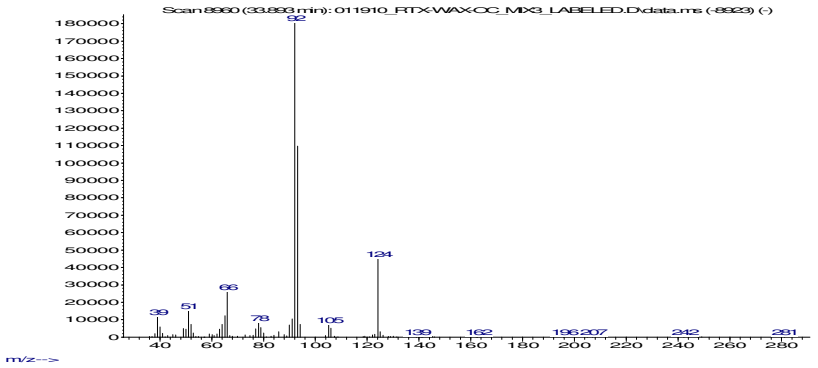
Response Factor of $^{13}C_2$-2-phenylethanol (February 10, 2010)

	Isotope	Unlabelled
Standard:	2-[1,2-$^{13}C_2$]-phenylethanol	2-phenylethanol
CAS:	NA	60-12-8
Mfg/Reference:	NA	Sigma, St Louis MO
No.; Catalog #; Batch#/Lot#:	NA	587; P6134-100ML; 066K1455
% Purity (by GC-FID)	99.9%	99.9%

Spectra



Unlabeled

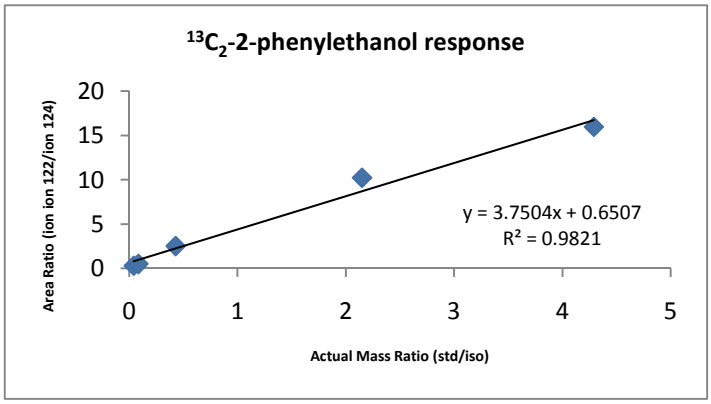


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		124	122
Mass Ratio:	0.0429	24609713	7223744
	0.0858	16300841	8039308
	0.429	2269087	5704476
	2.15	2411677	24658780
	4.29	3008295	48049274

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

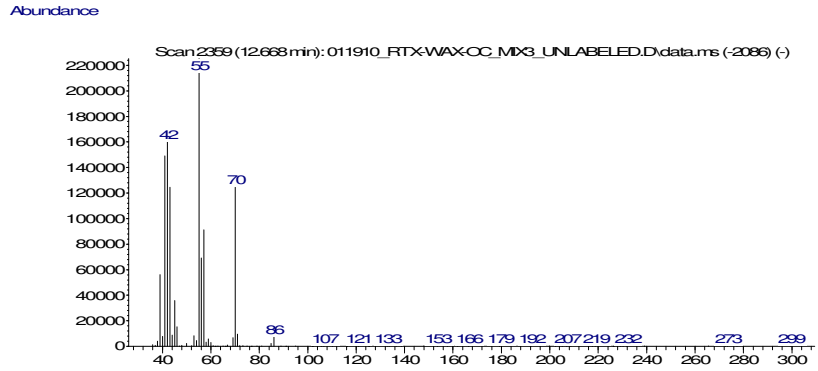


slope =	3.7504
response factor =	**0.267**

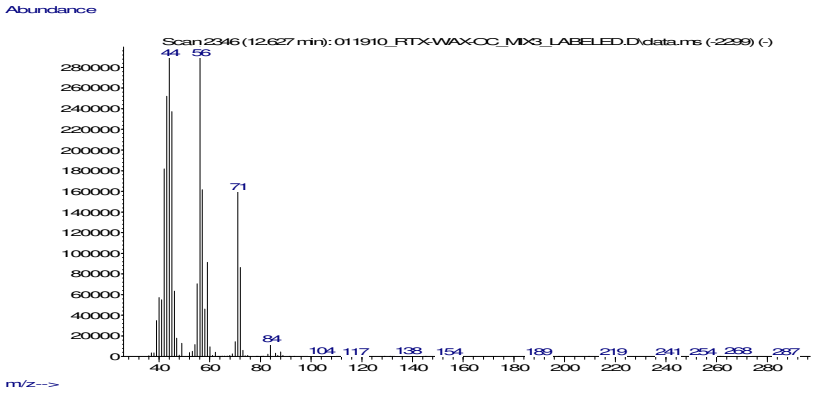
Response Factor of d_2-3-methyl-1-butanol (February 11, 2010)

	Isotope	Unlabeled
Standard:	3-methyl-[3,4-$^2H_2$]-1-butanol	3-methyl-1-butanol
CAS:	NA	123-51-3
Mfg/Reference:	NA	Fisher, Fair Lawn NJ
No.; Catalog #; Batch#/Lot#:	NA	188; A393-500; 952800
% Purity (by GC-FID)	99.9%	99.9%

Spectra



Unlabeled

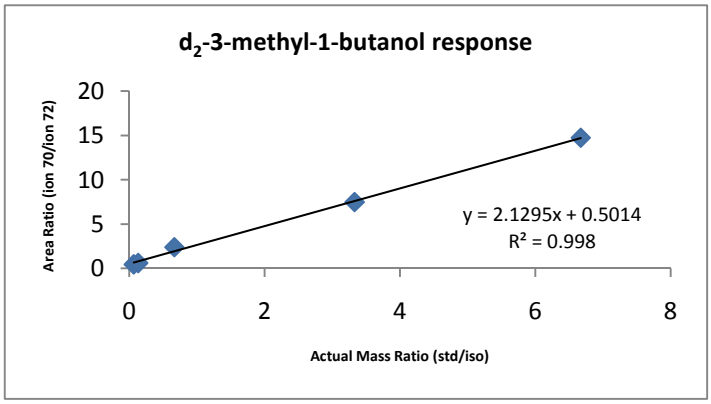


Labeled

Standard Curve

		Isotope	Unlabeled
Selected Ion:		72	70
Mass Ratio:	0.0429	24609713	7223744
	0.0858	16300841	8039308
	0.429	2269087	5704476
	2.15	2411677	24658780
	4.29	3008295	48049274

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

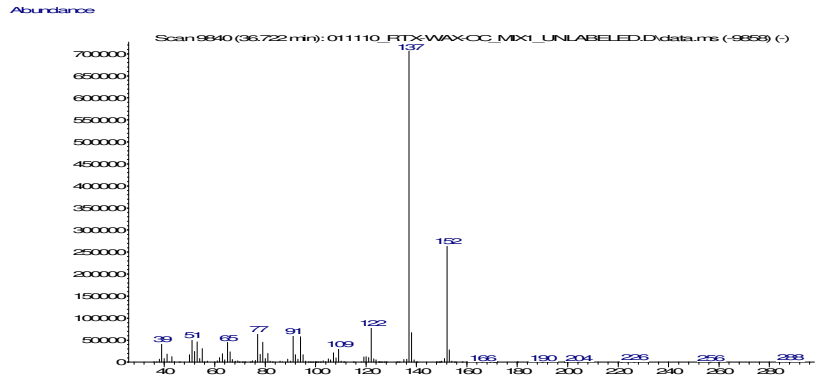


slope =	2.1295
response factor =	**0.450**

Response Factor of d_5-ethylguaiacol (January 12, 2010)

	Isotope	Unlabelled
Standard:	4-($^2H_5$-ethyl)-2-methoxyphenol	4-ethyl-2-methoxyphenol
CAS:	NA	2785-89-9
Mfg/Reference:	NA	Alfa Aesar (Lancaster, England)
No.; Catalog #; Batch#/Lot#:	NA	618; A14239; G1090C
% Purity (by GC-FID)	99.6%	97.7%

Spectra



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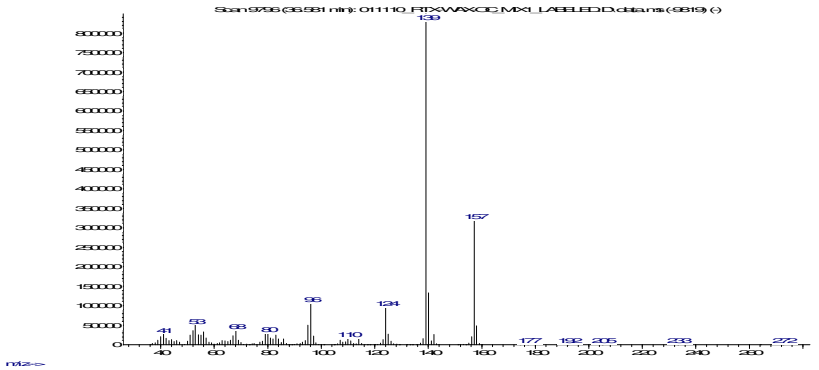
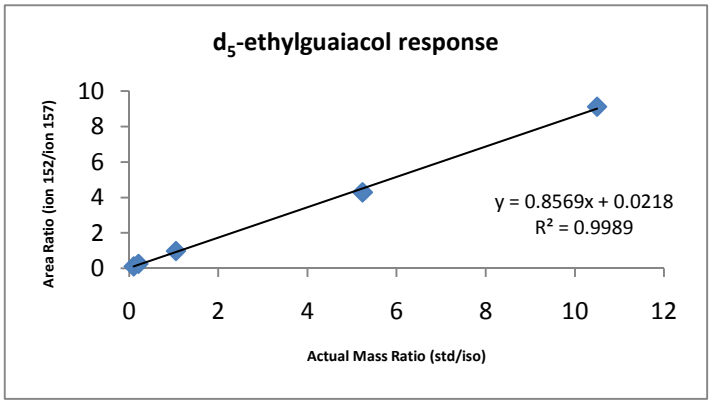


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		157	152
Mass Ratio:	0.104	128349526	14970591
	0.209	66477991	16917682
	1.05	16093133	15736910
	5.24	16751899	71939828
	10.5	14598091	133145411

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

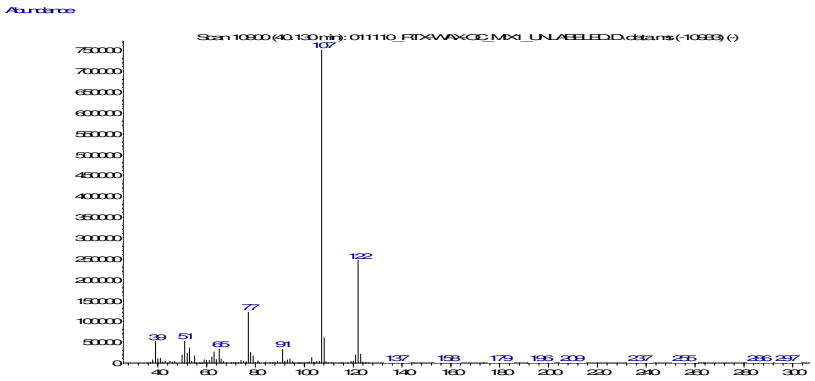


slope =	0.8569
response factor =	**1.17**

Response Factor of d_5-ethylphenol (January 12, 2010)

	Isotope	Unlabelled
Standard:	4-($^2H_5$-ethyl)-phenol	4-ethylphenol
CAS:	NA	123-07-9
Mfg/Reference:	NA	Sigma (St. Louis, MO)
No.; Catalog #; Batch#/Lot#:	NA	403; E44205-5G; 00313BH
% Purity (by GC-FID)	98.7%	98.5%

Spectra



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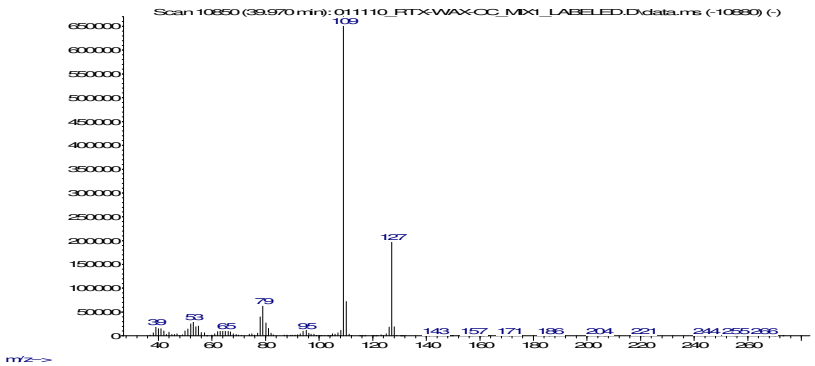
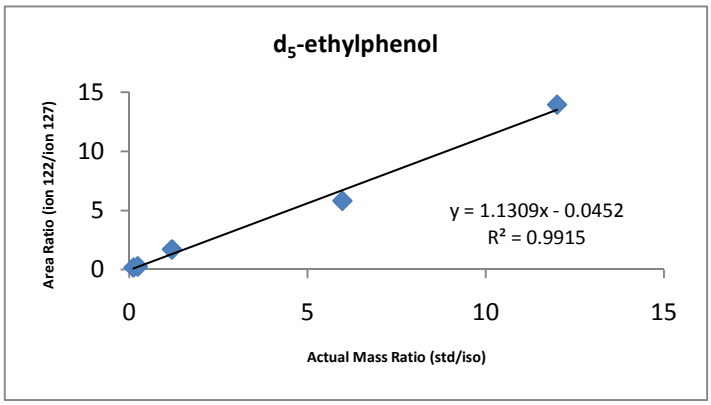


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		127	122
Mass Ratio:	0.120	83029105	13937742
	0.239	44791188	11494874
	1.20	8119354	13793843
	5.98	11035315	64102008
	12.0	8529758	118889994

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

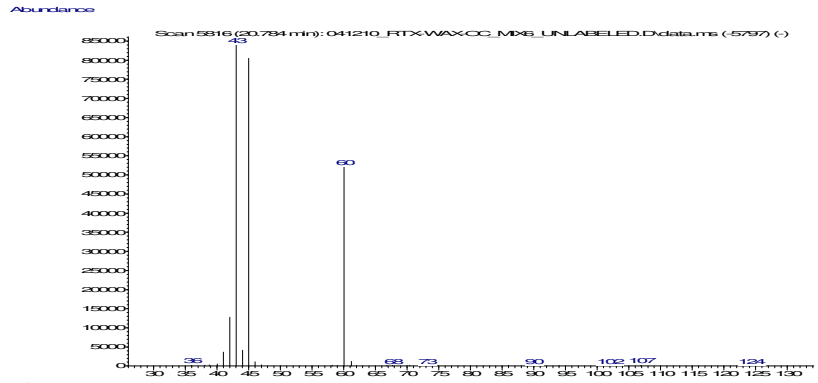


slope =	1.1309
response factor =	**0.884**

Response Factor of d_3-acetic acid (April 14, 2010)

	Isotope	Unlabelled
Standard:	d_3-acetic acid	acetic acid
CAS:	NA	?
Mfg/Reference:	CDN	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	?
% Purity (by GC-FID)	99.9%	99.99%

Spectra



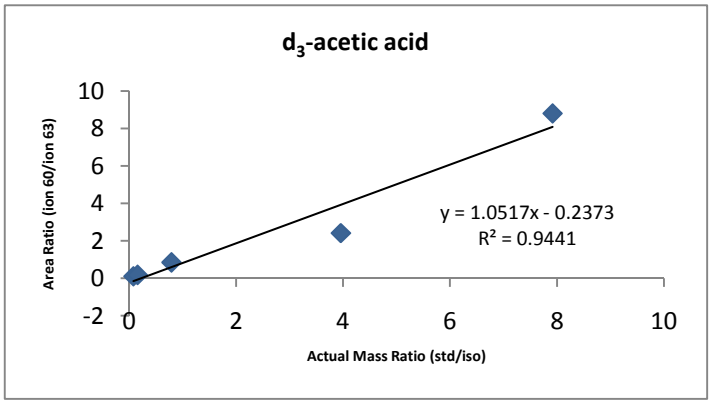
Unlabeled

Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		63	60
Mass Ratio:	0.079	21412845	2454518
	0.158	12372709	2288461
	0.791	3045854	2609695
	3.957	3516309	8504222
	7.915	2170325	19111545

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

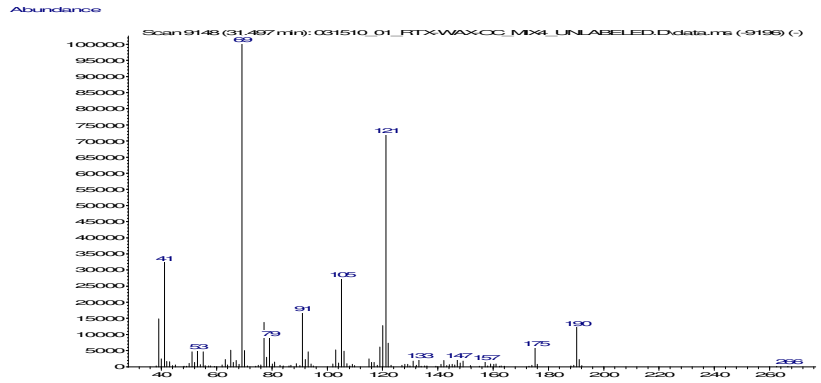


slope =	1.0517
response factor =	**0.9508**

Response Factor of d_4-β-damascenone (March 18, 2010)

	Isotope	Unlabeled
Standard:	d_4-β-damascenone	β-damascenone
CAS:	NA	
Mfg/Reference:	NA	Firmenich
No.; Catalog #; Batch#/Lot#:	NA	1075
% Purity (by GC-FID)	89.3%	95.01%

Spectra



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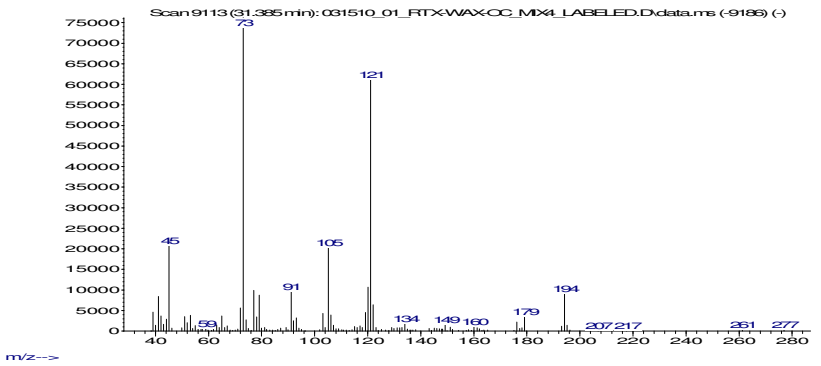
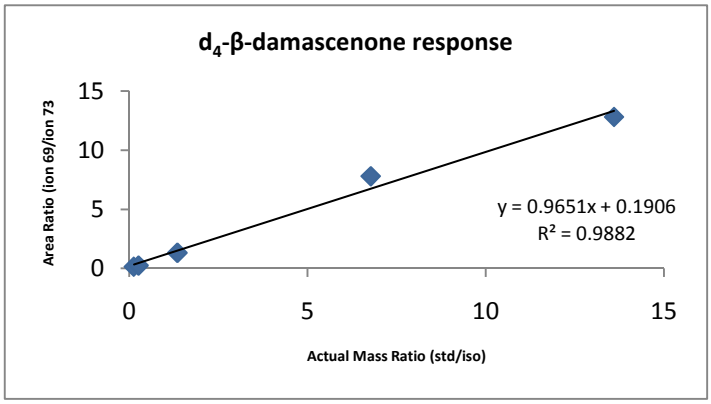


Labeled

Standard Curve

		Isotope	Unlabeled
Selected Ion:		73	69
Mass Ratio:	0.136	24289227	3642989
	0.271	11634560	2877943
	1.36	2668448	3527635
	6.78	2286627	17832144
	13.6	2146779	27497714

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

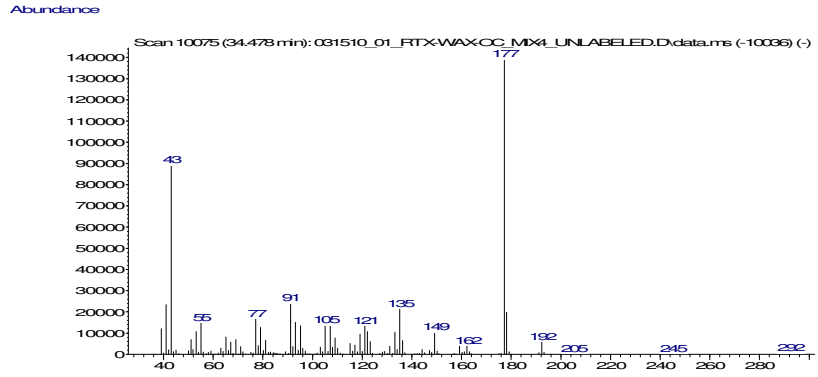


slope =	0.9651
response factor =	**1.036**

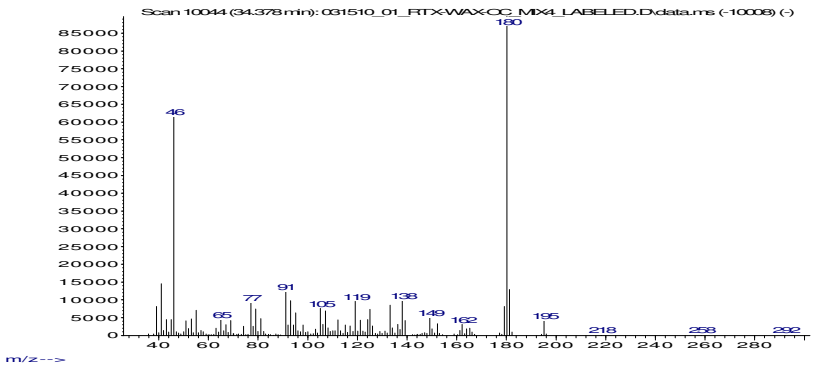
Response Factor of d_3-β-ionone (March 18, 2010)

	Isotope	Unlabeled
Standard:	d_3-β-ionone	β-ionone
CAS:	NA	79-77-6
Mfg/Reference:	NA	SAFC, St Louis, MO
No.; Catalog #; Batch#/Lot#:	NA	?; W259500; 07329KE
% Purity (by GC-FID)	90.43%	98.13%

Spectra



Unlabeled

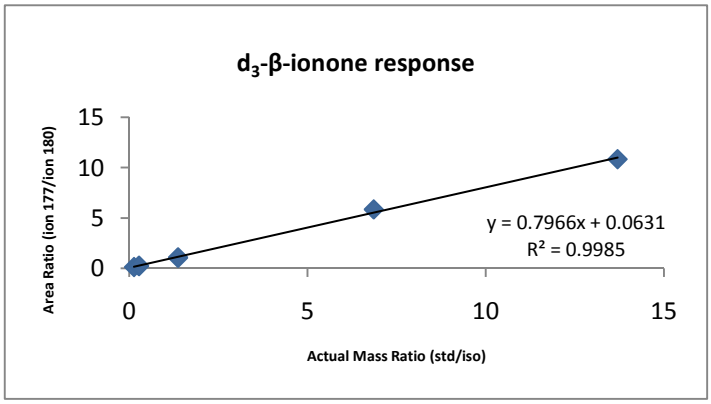


Labeled

Standard Curve

		Isotope	**Unlabeled**
Selected Ion:		180	177
Mass Ratio:	0.137	32147432	4309128
	0.275	16515025	4013060
	1.37	3298624	3506900
	6.86	3642672	21290078
	13.7	3099958	33565662

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

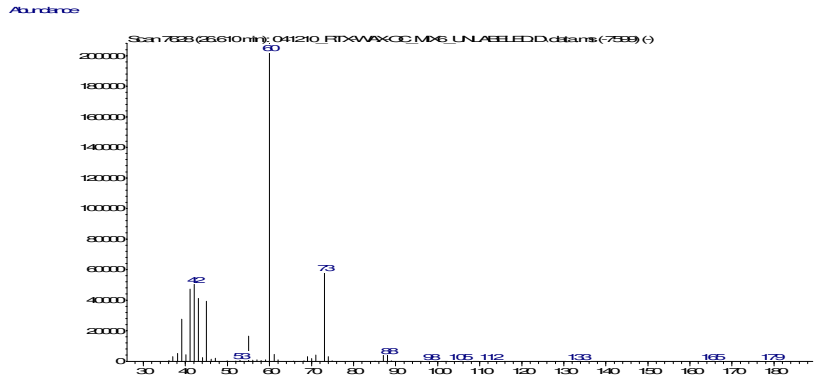


slope =	0.7966
response factor =	**1.255**

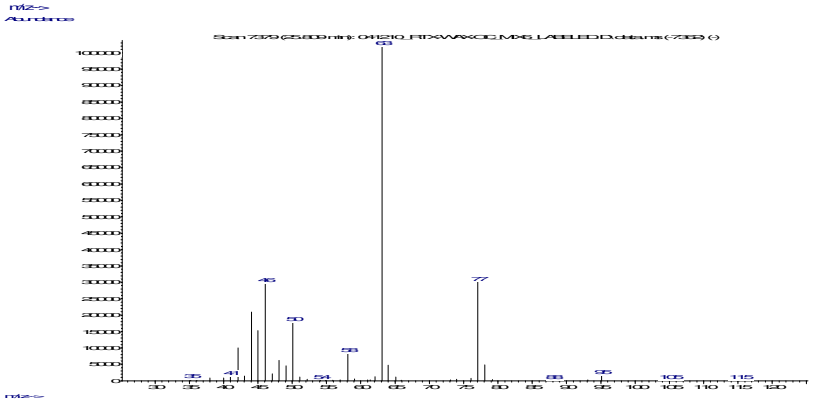
Response Factor of d_7-butyric acid (April 14, 2010)

	Isotope	Unlabelled
Standard:	d_7-butyric acid	butyric acid
CAS:	NA	107-92-6
Mfg/Reference:	CDN	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	641; B10,350-0; 11503CI
% Purity (by GC-FID)	99.9%	99.99%

Spectra



Unlabeled

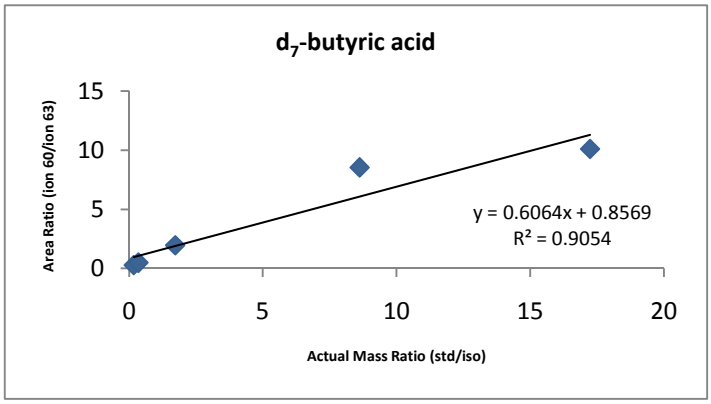


Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		63	60
Mass Ratio:	0.172	41484079	11243719
	0.345	23825492	11314104
	1.723	3767472	7314004
	8.615	4245432	36233346
	17.23	7252846	73201648

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

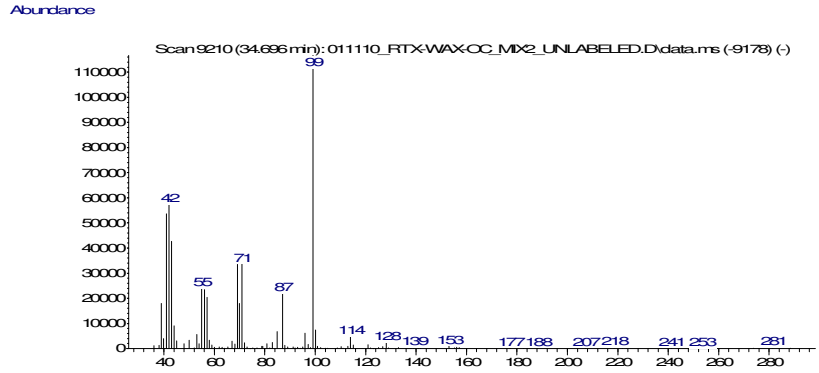


slope =	0.6064
response factor =	**1.6491**

Response Factor of d_2-*cis*-whiskey lactone (April 7, 2010)

	Isotope	Unlabelled
Standard:	(3*S*, 4*S*)-*cis*-[$^2H_2$-2,3]-whiskey lactone	(3*S*, 4*S*)-*cis*-whiskey lactone
CAS:	NA	39212-23-2
Mfg/Reference:	NA	SAFC, St Louis MO
No.; Catalog #; Batch#/Lot#:	NA	666
% Purity (by GC-FID)	99.29% (44.5% trans, 48.9% cis)	99% (52.2% trans, 47.8% cis)

Spectra



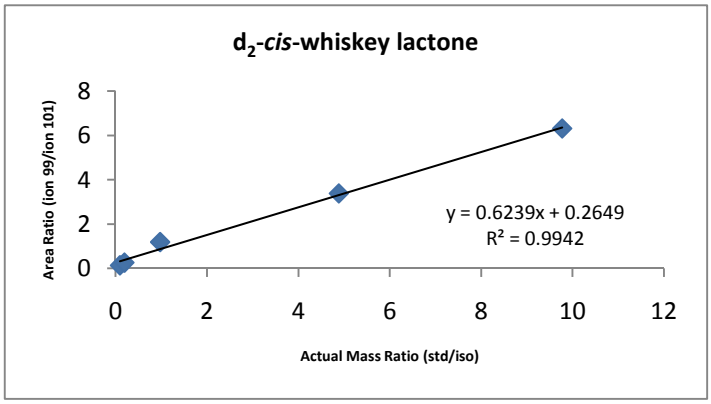
Unlabeled

Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		101	99
Mass Ratio:	0.098	31429558	4257746
	0.196	12485971	3238966
	0.976	3205438	3804255
	4.888	4594669	15524783
	9.775	4732973	29840059

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

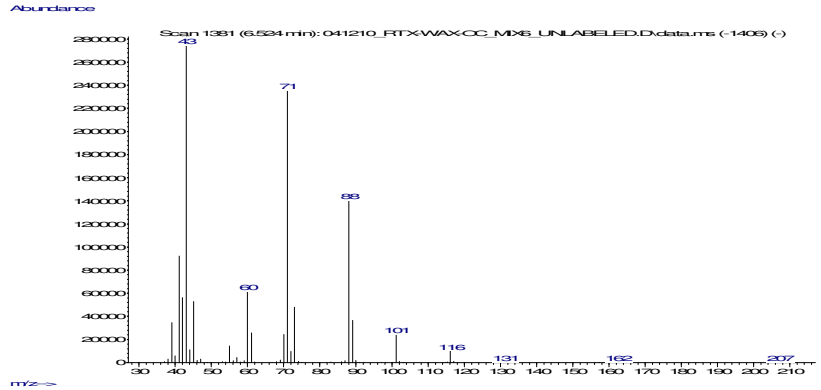


slope =	0.6239
response factor =	**1.603**

Response Factor of d_7-ethyl butyrate (April 14, 2010)

	Isotope	Unlabelled
Standard:	ethyl [$^2H_7$]-butyrate	ethyl butyrate
CAS:	NA	105-54-4
Mfg/Reference:	CDN	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	283; E1,570-1; 07721KQ
% Purity (by GC-FID)	99%	99.99%

Spectra



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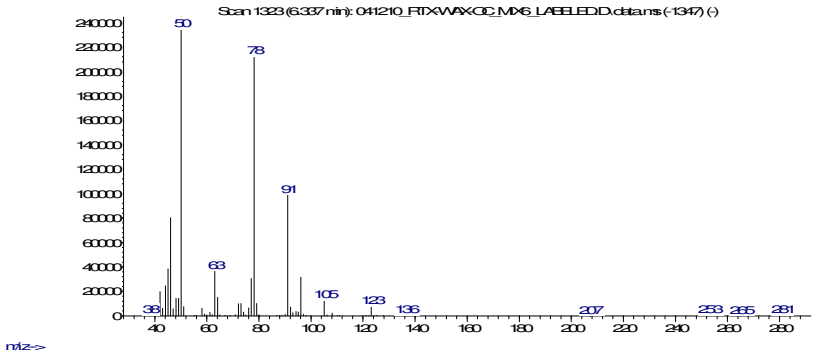
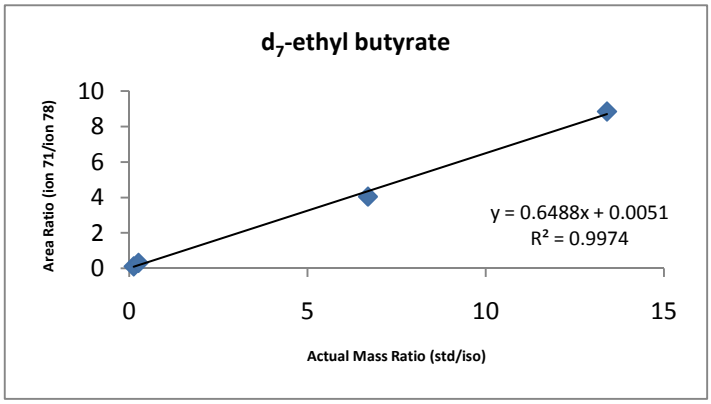


Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		78	71
Mass Ratio:	0.134	32979111	3784987
	0.268	17637458	5496163
	1.340	NA	NA
	6.700	5570915	22545502
	13.40	4401666	38946092

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

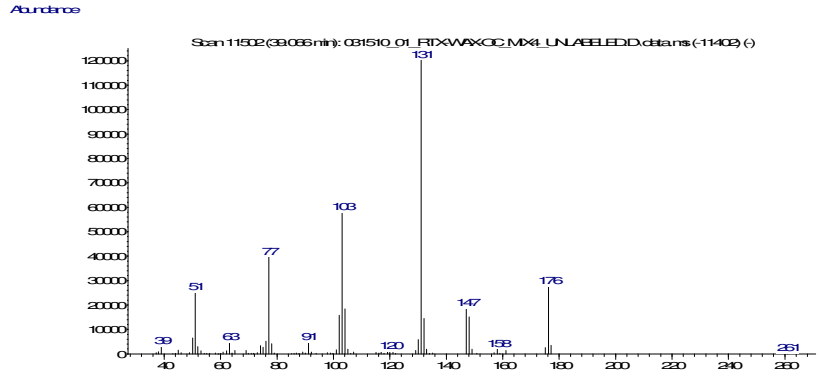


slope =	0.6488
response factor =	**1.5413**

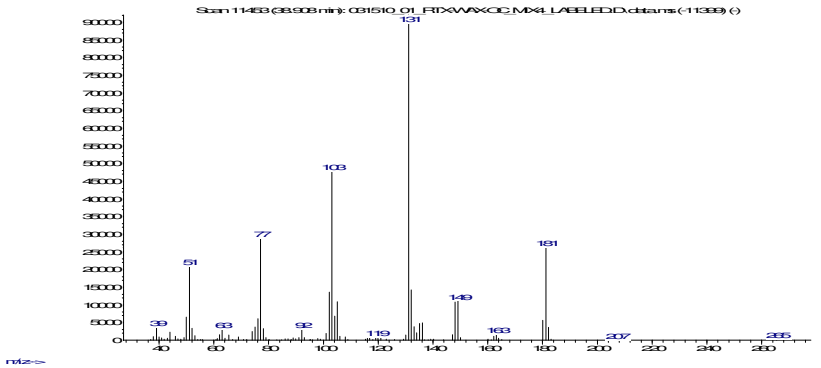
Response Factor of d_5-ethyl cinnamate (March 31, 2010)

	Isotope	Unlabeled
Standard:	d_5- ethyl cinnamate	ethyl cinnamate
CAS:	NA	103-36-6
Mfg/Reference:	NA	Alfa Aesar, Lancaster, UK
No.; Catalog #; Batch#/Lot#:	NA	?; A12906; 10143034
% Purity (by GC-FID)	94.4%	99.91%

Spectra



Unlabeled

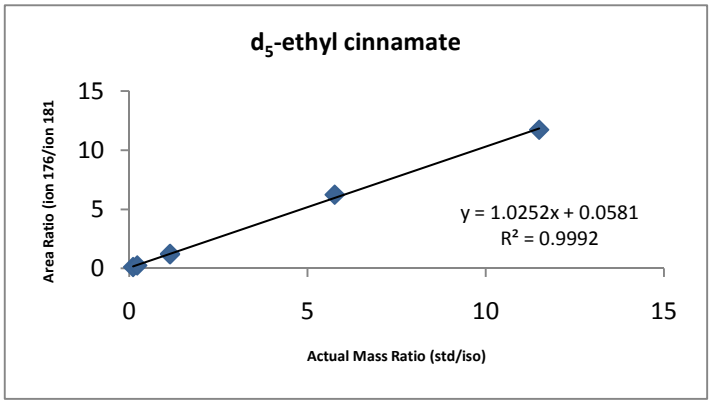


Labeled

Standard Curve

		Isotope	**Unlabeled**
Selected Ion:		181	176
Mass Ratio:	0.115	9590280	1147905
	0.231	4813117	1192134
	1.15	711553	860642
	5.77	807227	5027198
	11.5	707476	8294846

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

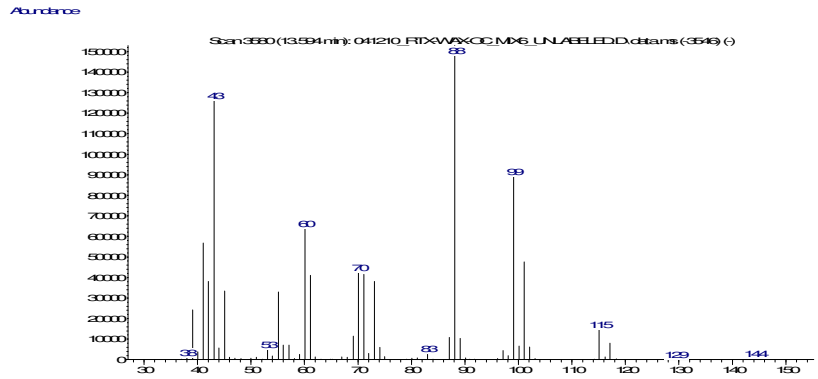


slope =	1.0252
response factor =	**0.975**

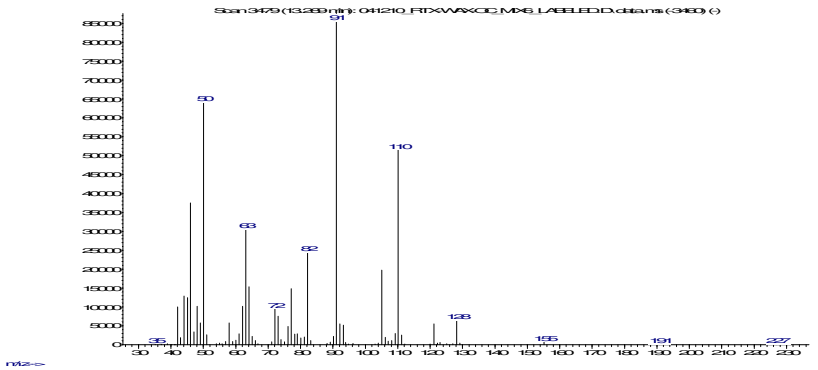
Response Factor of d_{11}-ethyl hexanoate (April 14, 2010)

	Isotope	Unlabelled
Standard:	d_{11}-ethyl hexanoate	ethyl hexanoate
CAS:	NA	123-66-0
Mfg/Reference:	CDN	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	287; 14,896-2; 15201MR
% Purity (by GC-FID)	99%	99.99%

Spectra



Unlabeled

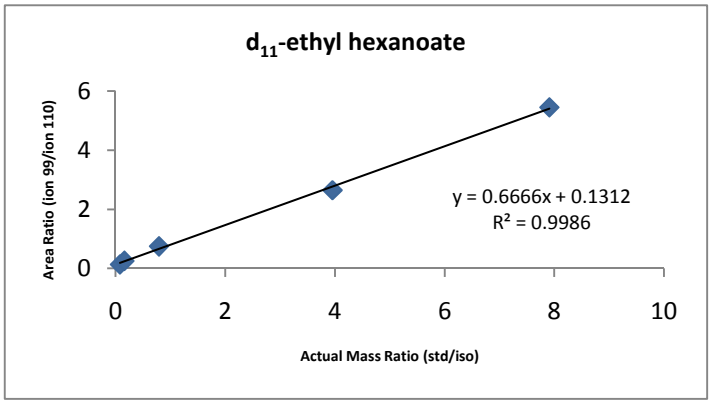


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		110	99
Mass Ratio:	0.079	17435238	2405969
	0.158	10192936	2640134
	0.791	3428474	2571604
	3.954	3119337	8263279
	7.908	2747531	14981388

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

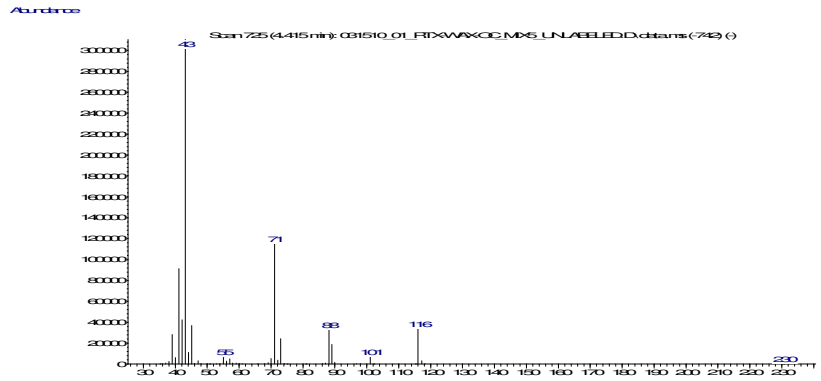


slope =	1.5002
response factor =	**0.6666**

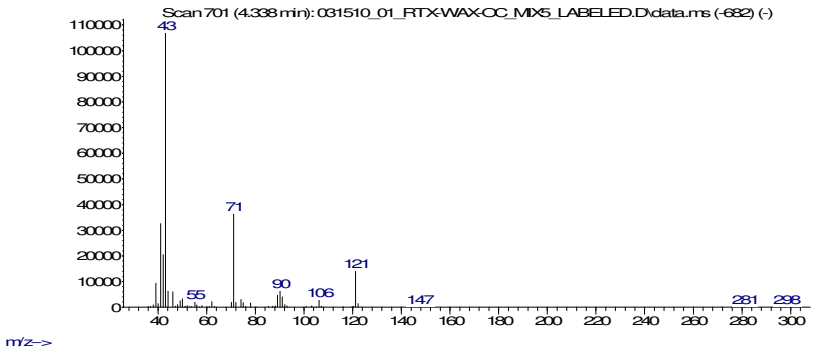
Response Factor of d_5-ethyl isobutyrate (April 1, 2010)

	Isotope	Unlabeled
Standard:	d_5- ethyl isobutyrate	ethyl isobutyrate
CAS:	NA	97-62-1
Mfg/Reference:	NA	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	?; 246085-100ML; 01319ME
% Purity (by GC-FID)	99.90%	99.99%

Spectra



Unlabeled

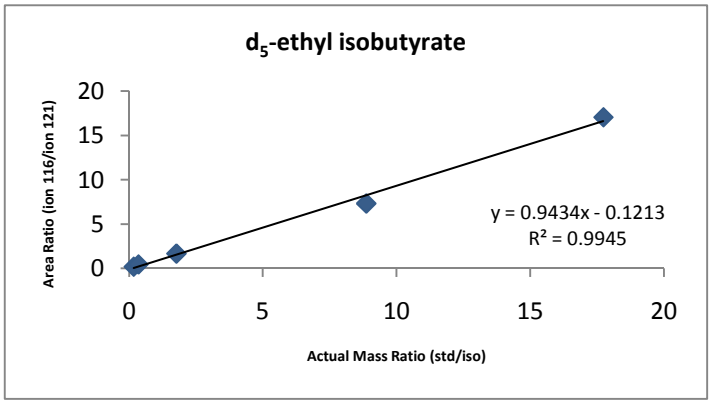


Labeled

Standard Curve

		Isotope	**Unlabeled**
Selected Ion:		121	116
Mass Ratio:	0.177	3246901	622945
	0.355	1238558	541707
	1.773	383400	636428
	8.867	414785	3036454
	17.73	240637	4103008

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

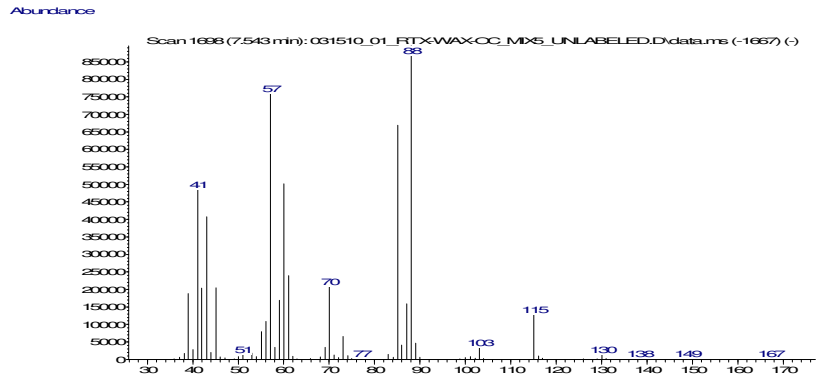


slope =	0.9434
response factor =	**1.060**

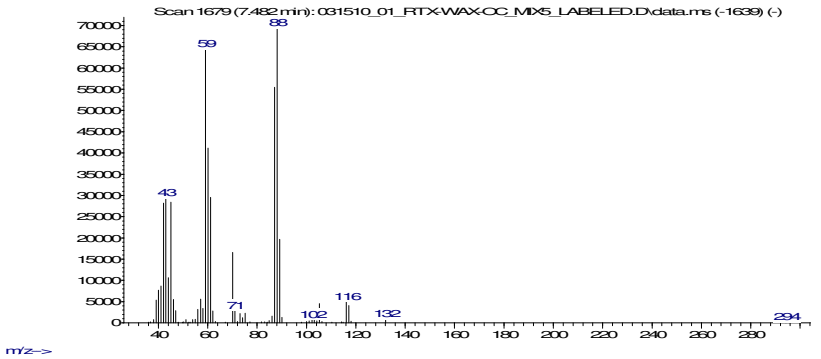
Response Factor of d_2-ethyl isovalerate (April 1, 2010)

	Isotope	Unlabeled
Standard:	d_2- ethyl isovalerate	ethyl isovalerate
CAS:	NA	108-64-5
Mfg/Reference:	NA	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	?; 11283-100ML; 07131AE
% Purity (by GC-FID)	97.50%	99.99%

Spectra



Unlabeled

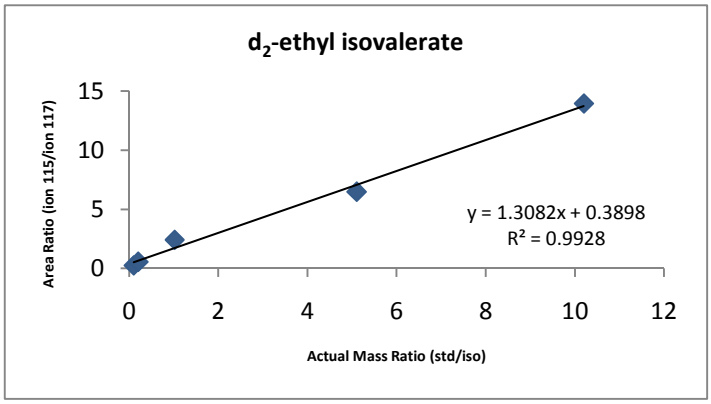


Labeled

Standard Curve

		Isotope	**Unlabeled**
Selected Ion:		117	115
Mass Ratio:	0.102	1488845	394071
	0.204	582079	329959
	1.020	162410	393501
	5.102	206592	1341703
	10.20	148733	2075338

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

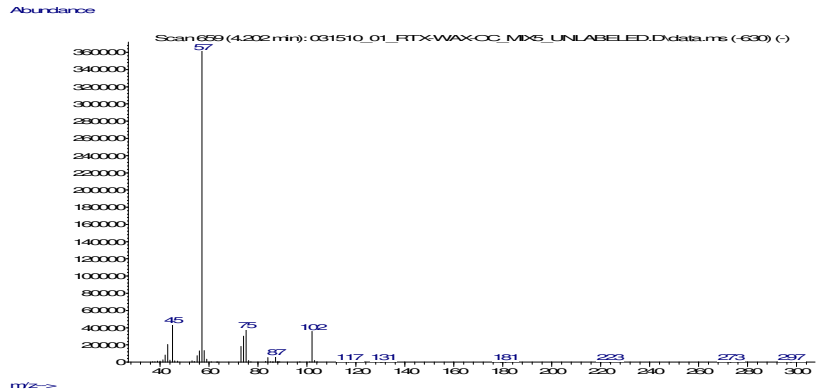


slope =	1.3082
response factor =	**0.7644**

Response Factor of d_5-ethyl isobutyrate (vs ethyl propanoate) (March 31, 2010)

	Isotope	Unlabeled
Standard:	d_5- ethyl isobutyrate	ethyl propanoate
CAS:	NA	105-37-3
Mfg/Reference:	NA	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	293; 11,230-5; 07617KN
% Purity (by GC-FID)	99.90%	99.99%

Spectra



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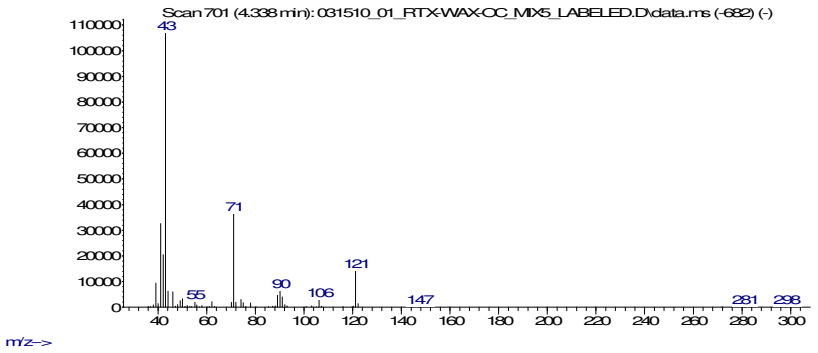
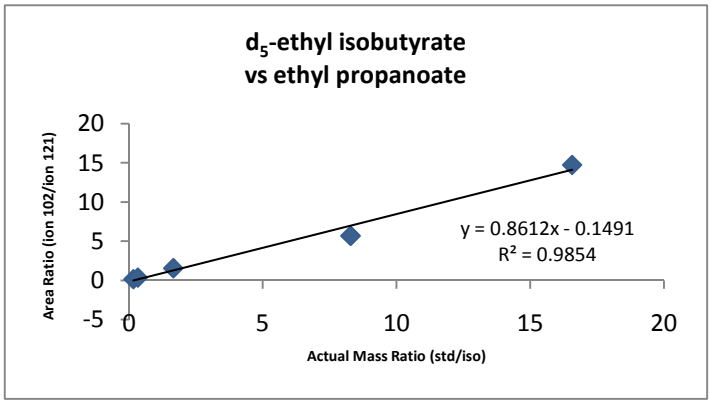


Labeled

Standard Curve

		Isotope	**Unlabeled**
Selected Ion:		121	102
Mass Ratio:	0.166	3246901	538803
	0.331	1238558	453148
	1.656	383400	596051
	8.281	414785	2360359
	16.56	240637	3542985

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

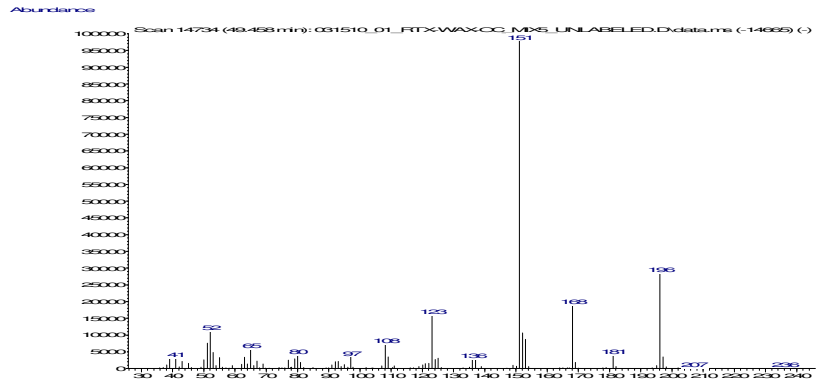


slope =	0.8612
response factor =	**1.161**

Response Factor of d_5-ethyl vanillate (March 31, 2010)

	Isotope	Unlabeled
Standard:	d_5- ethyl vanillate	ethyl vanillate
CAS:	NA	617-05-0
Mfg/Reference:	NA	Alfa Aesar, Lancaster, UK
No.; Catalog #; Batch#/Lot#:	NA	?; L05798; 10108450
% Purity (by GC-FID)	96.6%	99.90%

Spectra



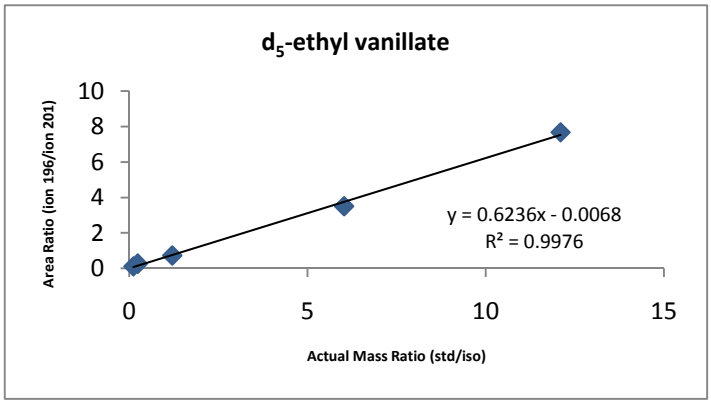
Unlabeled

Labeled

Standard Curve

		Isotope	Unlabeled
Selected Ion:		201	196
Mass Ratio:	0.121	10502300	1163215
	0.241	6862422	1771205
	1.21	1647398	1185598
	6.03	1810633	6331750
	12.1	1605010	12305193

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

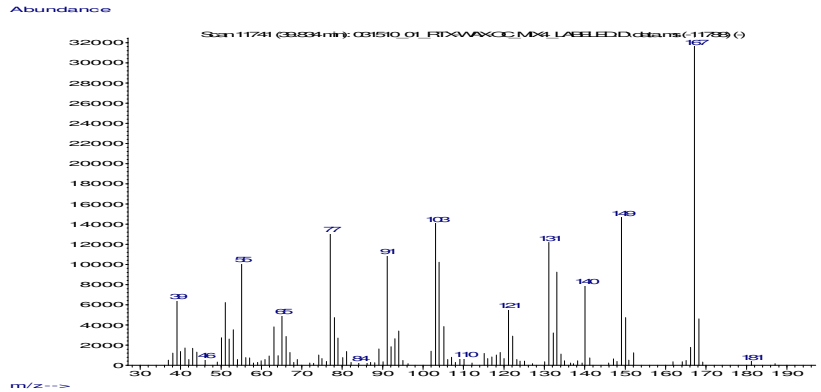


slope =	0.6236
response factor =	**1.603**

Response Factor of d_3-eugenol (March 18, 2010)

	Isotope	Unlabeled
Standard:	d_3- eugenol	eugenol
CAS:	NA	97-53-0
Mfg/Reference:	NA	Aldrich, Milwaukee, WI
No.; Catalog #; Batch#/Lot#:	NA	640; E5,179-1; 01114CV
% Purity (by GC-FID)	80%	98.8%

Spectra



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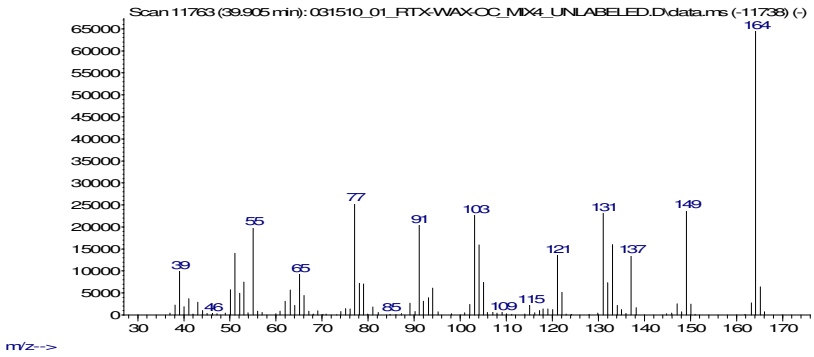
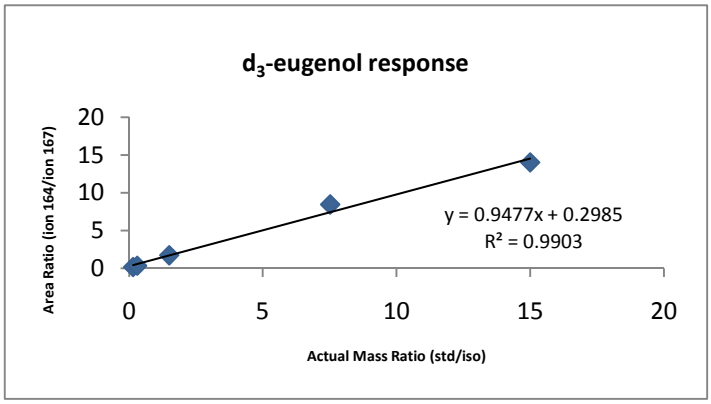


Labeled

Standard Curve

		Isotope	**Unlabeled**
Selected Ion:		167	164
Mass Ratio:	0.150	12300051	2124705
	0.301	6188367	2062207
	1.50	1261854	2171162
	7.52	1130052	9547120
	15.0	835279	11701150

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

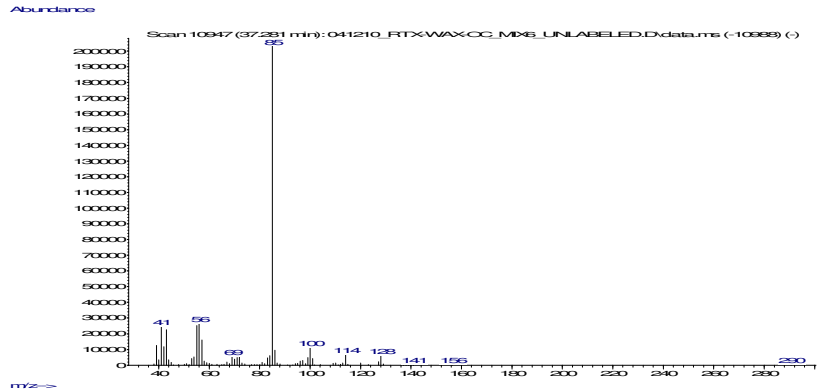


slope =	0.9477
response factor =	**1.055**

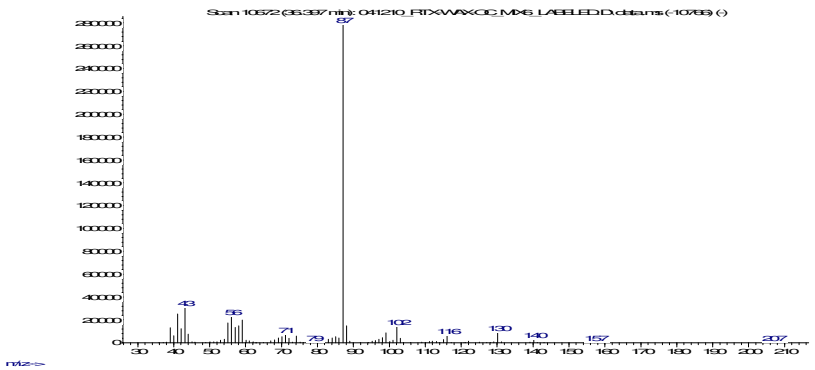
Response Factor of d_2-γ-nonalactone (April 14, 2010)

	Isotope	Unlabelled
Standard:	d_2-γ-nonalactone	γ-nonalactone
CAS:	NA	104-61-0
Mfg/Reference:	NA	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	438; W27810-6; 07001HD
% Purity (by GC-FID)	92%	99%

Spectra



Unlabeled

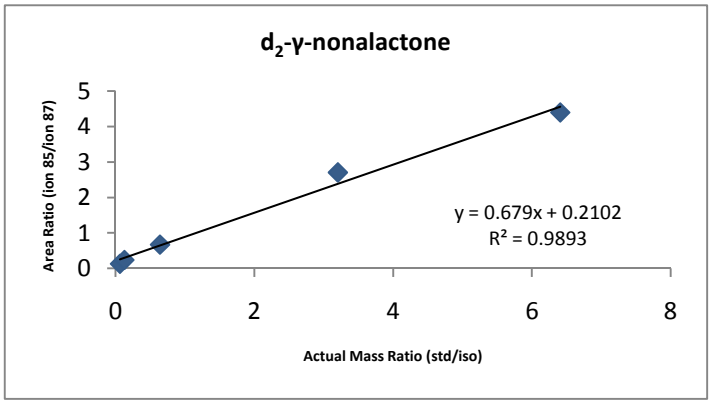


Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		87	85
Mass Ratio:	0.064	103459030	13266076
	0.128	55942352	13290255
	0.641	20427465	13682744
	3.203	16446444	44507011
	6.406	18436954	81108918

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

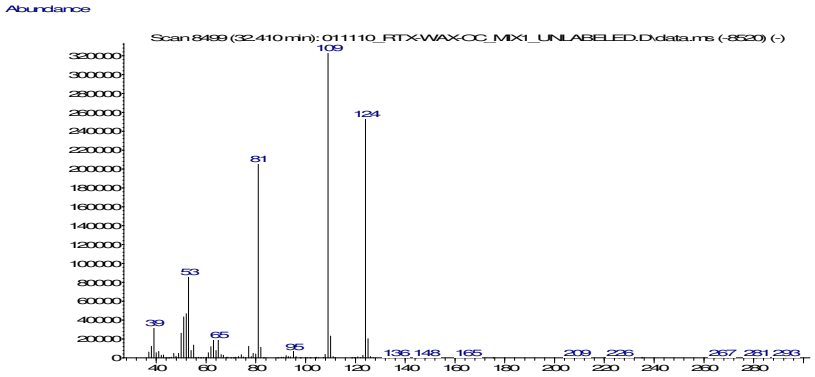


slope =	0.679
response factor =	**1.473**

Response Factor of d_3-Guaiacol (January 12, 2010)

	Isotope	Unlabelled
Standard:	2-($^2H_3$-methoxy)-phenol	2-methoxyphenol
CAS:	74495-69-5	90-05-1
Mfg/Reference:	CDN (Quebec, Canada)	Sigma (St. Louis, MO)
No.; Catalog #; Batch#/Lot#:	ISO-9; D-5968; W321P3	617; G-5502; 119F3505
% Purity (by GC-FID)	99.9%	99.9%

Spectra



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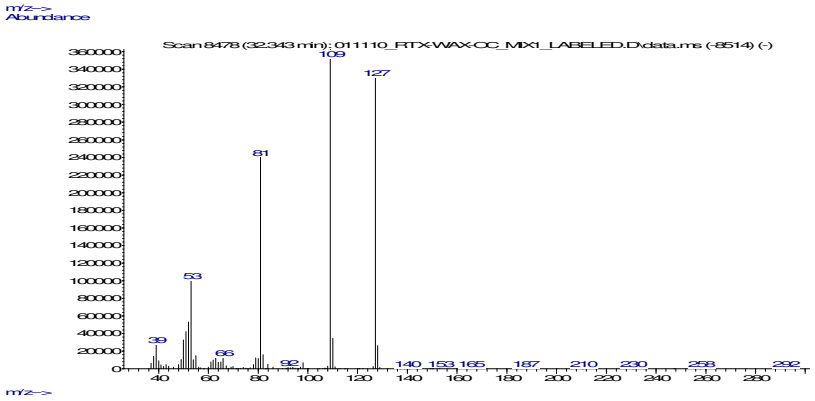
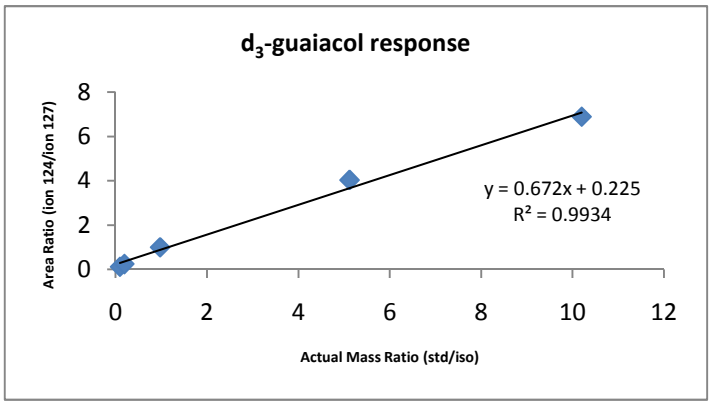


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		127	124
Mass Ratio:	0.0976	132404300	15442473
	0.195	67362208	16108411
	0.976	14919349	14859767
	5.12	14919349	66777745
	10.2	17153680	118155185

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

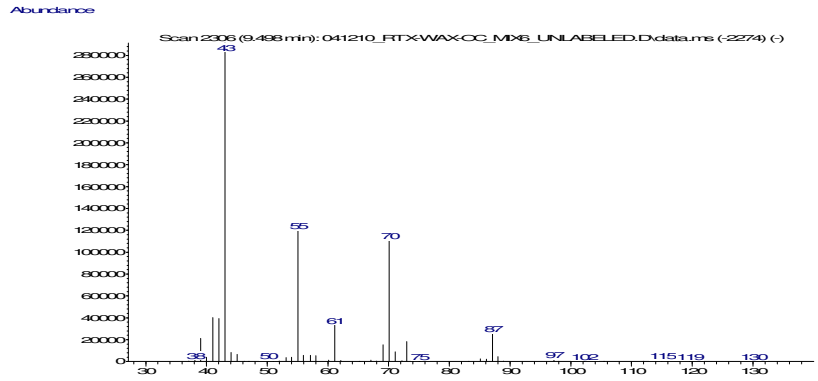


slope =	0.672
response factor =	**1.49**

Response Factor of d_2-isoamyl acetate (April 14, 2010)

	Isotope	Unlabelled
Standard:	d_2-isoamyl acetate	isoamyl acetate
CAS:	NA	123-92-2
Mfg/Reference:	NA	Fluka, Switzerland
No.; Catalog #; Batch#/Lot#:	NA	?; 79857; 1393547
% Purity (by GC-FID)	98.75%	99.7%

Spectra



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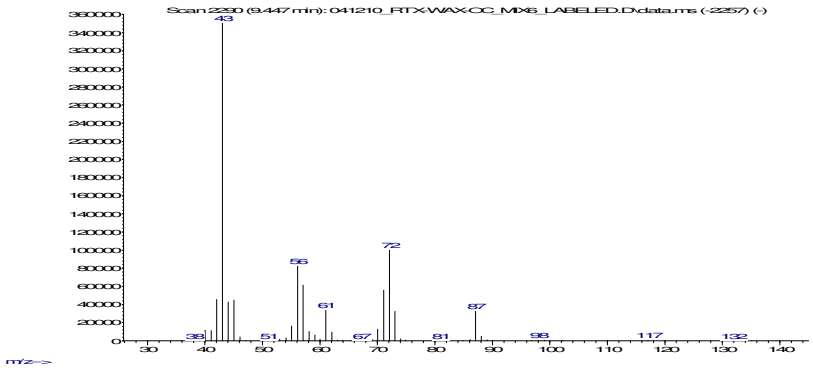
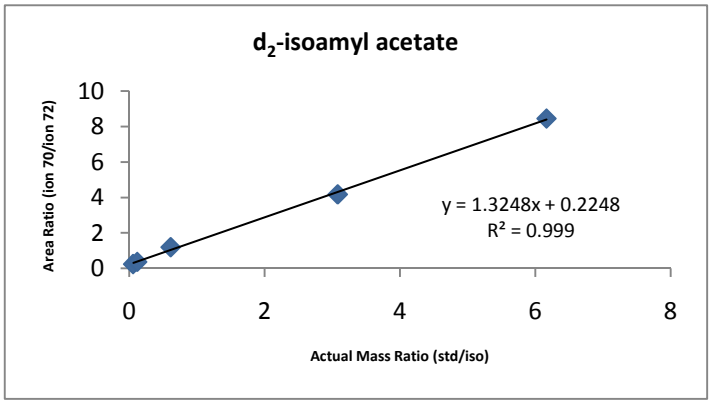


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		72	70
Mass Ratio:	0.062	24835644	6138850
	0.123	12907490	4781314
	0.617	2558369	3072933
	3.083	2713022	11327880
	6.166	2664871	22506511

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

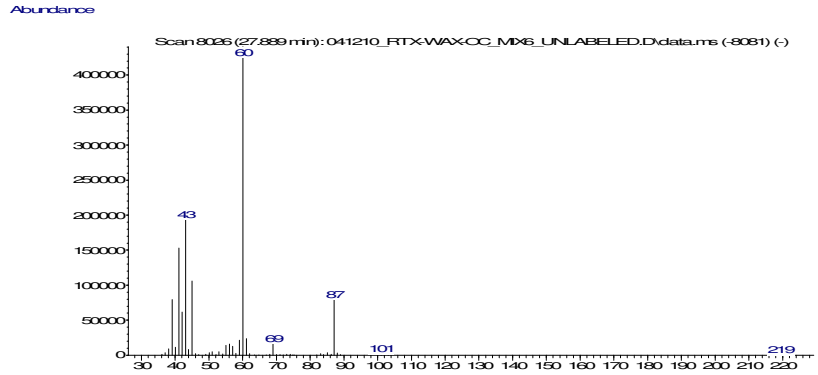


slope =	1.3248
response factor =	**0.7548**

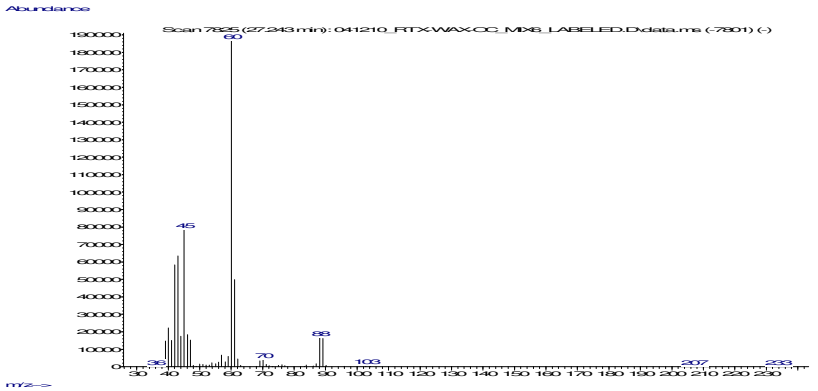
Response Factor of d_2-isovaleric acid (April 14, 2010)

	Isotope	Unlabelled
Standard:	d_2-isovaleric acid	isovaleric acid
CAS:	NA	503-74-2
Mfg/Reference:	NA	Aldrich, St Louis MO
No.; Catalog #; Batch#/Lot#:	NA	?; 129542-100ML; MKBB4414
% Purity (by GC-FID)	98.4%	99%

Spectra



Unlabeled

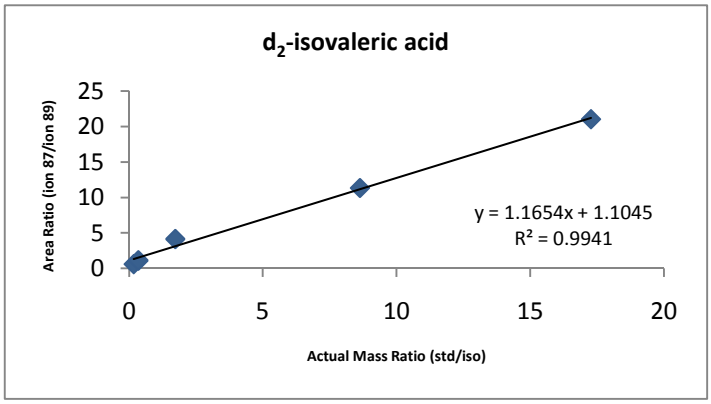


Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		89	87
Mass Ratio:	0.173	4973267	3129408
	0.345	2970506	3370770
	1.727	631355	2626982
	8.633	658494	7477719
	17.27	705812	14854606

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

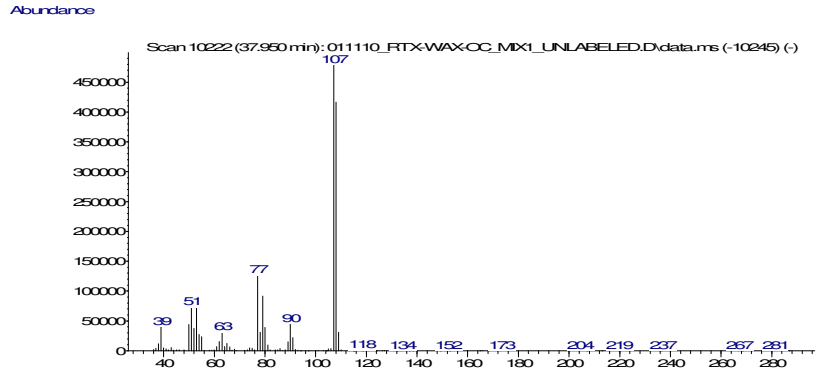


slope =	1.1654
response factor =	**0.8581**

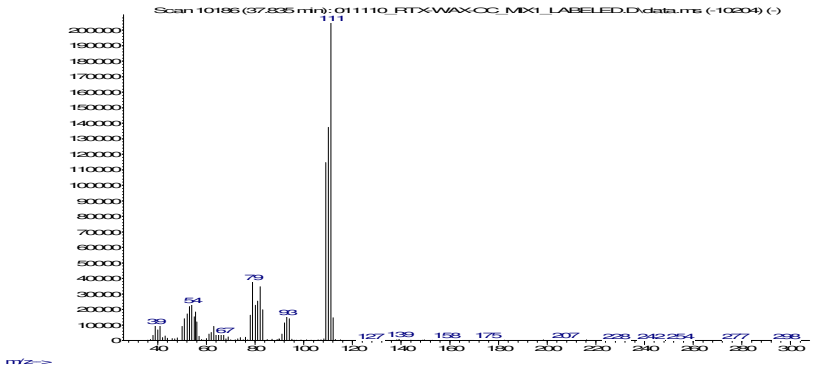
Response Factor of d_3-*p*-cresol (January 12, 2010)

	Isotope	Unlabelled
Standard:	4-($^2H_3$-methyl)-phenol	4-methylphenol (*p*-cresol)
CAS:	108561-00-8	106-44-5
Mfg/Reference:	CDN (Quebec, Canada)	Aldrich (Milwaukee, WI)
No.; Catalog #; Batch#/Lot#:	ISO-5; D-5638; R653P1	425; C8,575-1; 09410PI
% Purity (by GC-FID)	99.3%	99.9%

Spectra



Unlabeled

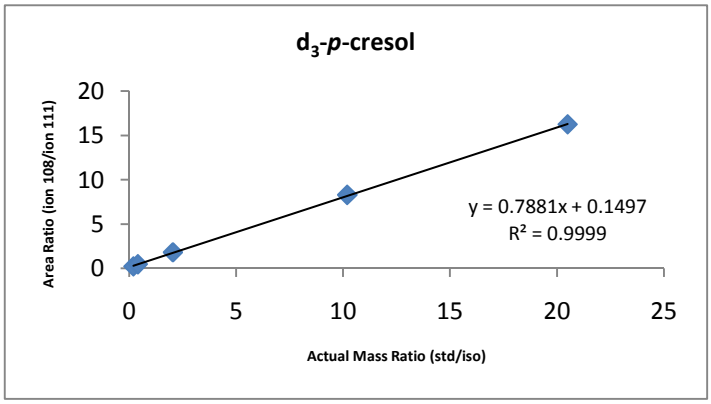


Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		111	108
Mass Ratio:	0.205	94929880	21481512
	0.410	49813489	23059432
	2.05	12258698	22186772
	10.2	12816042	106319570
	20.5	10928800	177588483

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

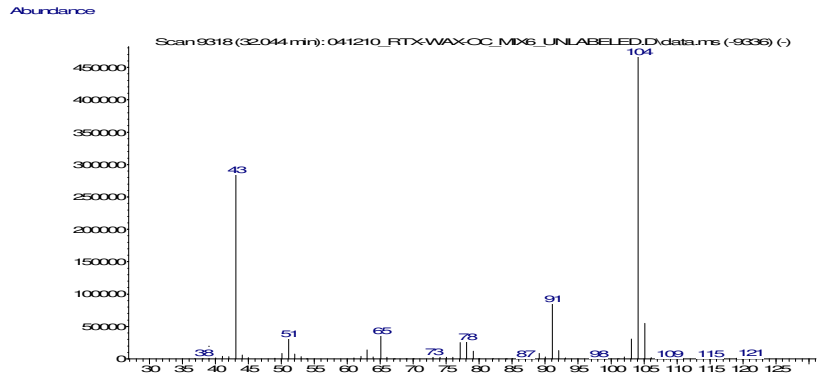


slope =	0.7881
response factor =	**1.27**

Response Factor of $^{13}C_2$-phenethyl acetate (April 14, 2010)

	Isotope	Unlabelled
Standard:	$^{13}C_2$-phenethyl acetate	phenethyl acetate
CAS:	NA	103-45-7
Mfg/Reference:	NA	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	427; 29058-0; ?
% Purity (by GC-FID)	99%	99%

Spectra



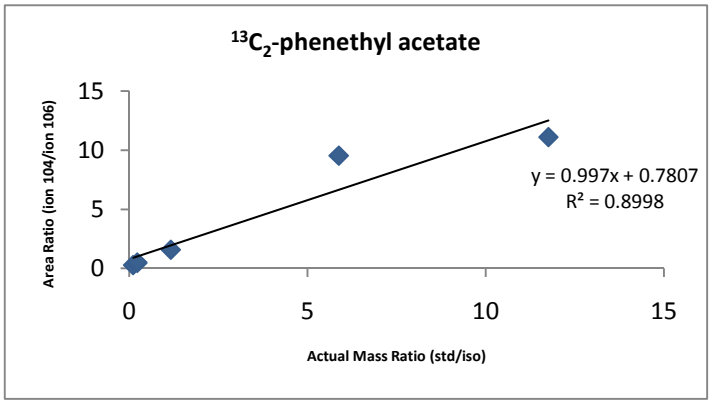
Unlabeled

Labeled

Standard Curve

		Isotope	Unlabelled
Selected Ion:		106	104
Mass Ratio:	0.118	62952104	18241559
	0.235	34895914	17106128
	1.176	9301561	14719105
	5.882	6171665	58923584
	11.76	9460722	105089499

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

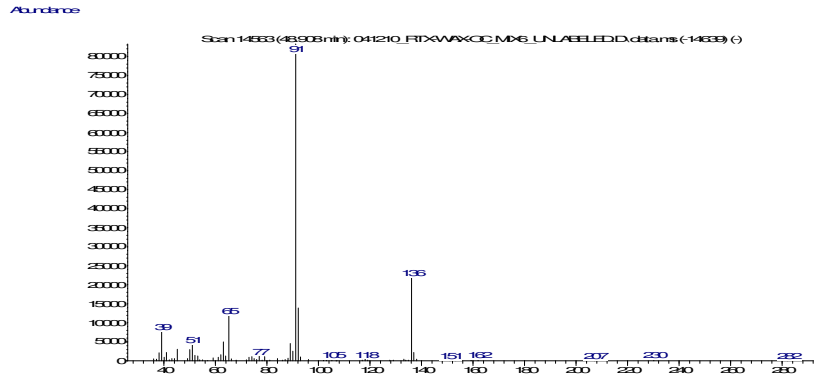


slope =	0.997
response factor =	**1.003**

Response Factor of $^{13}C_2$-phenylacetic acid (April 14, 2010)

	Isotope	Unlabelled
Standard:	$^{13}C_2$-phenylacetic acid	phenylacetic acid
CAS:	NA	103-82-2
Mfg/Reference:	Isotec	Aldrich, Milwaukee WI
No.; Catalog #; Batch#/Lot#:	NA	877; P16621-5G; 02804ED
% Purity (by GC-FID)	99.9%	99%

Spectra



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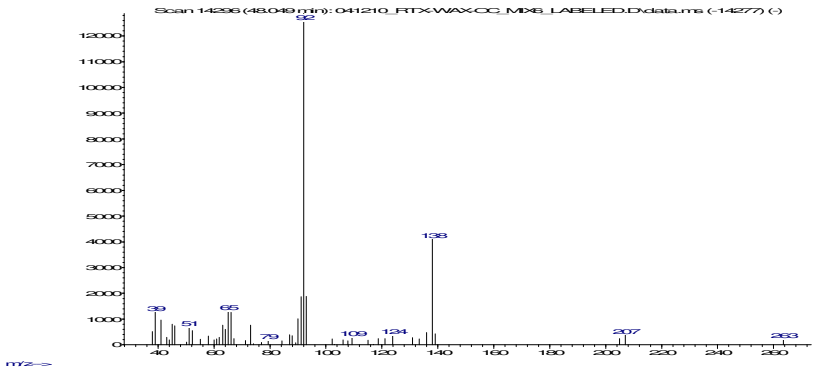
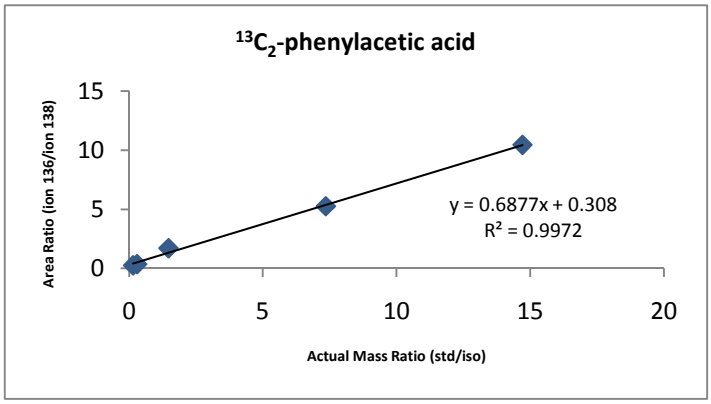


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		138	136
Mass Ratio:	0.147	13620514	3528792
	0.294	7064444	2557543
	1.471	1447080	2470400
	7.355	2199486	11560227
	14.71	2324643	24280361

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

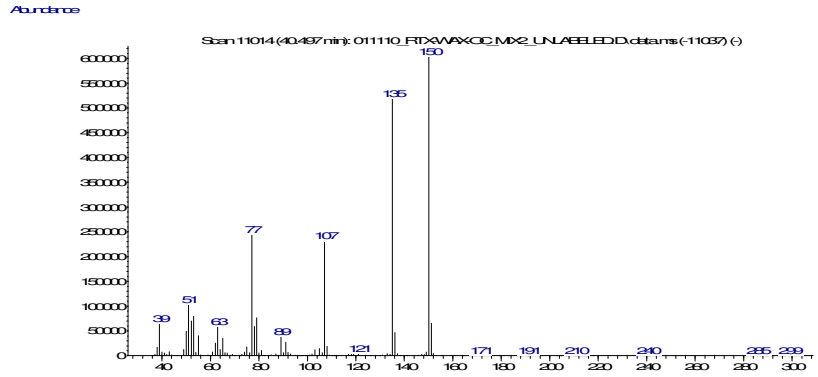


slope =	0.6877
response factor =	**1.4541**

Response Factor of d_3-*p*-vinylguaiacol (January 13, 2010)

	Isotope	Unlabelled
Standard:	2-[$^2H_3$]-methoxy-4-vinylphenol	2-methoxy-4-vinylphenol
CAS:	NA	NA
Mfg/Reference:	NA	NA
No.; Catalog #; Batch#/Lot#:	NA	NA
% Purity (by GC-FID)	99.9%	99%

Spectra



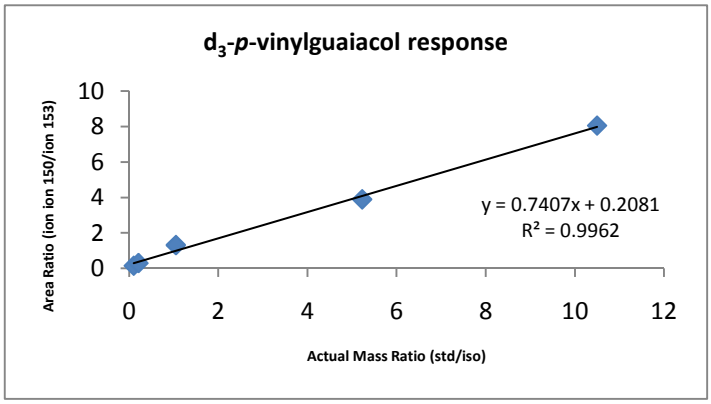
Unlabeled

Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		153	150
Mass Ratio:	0.105	119464885	17993321
	0.209	52778549	15624460
	1.05	14207852	18641779
	5.23	19382148	75475676
	10.5	16324776	131392125

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

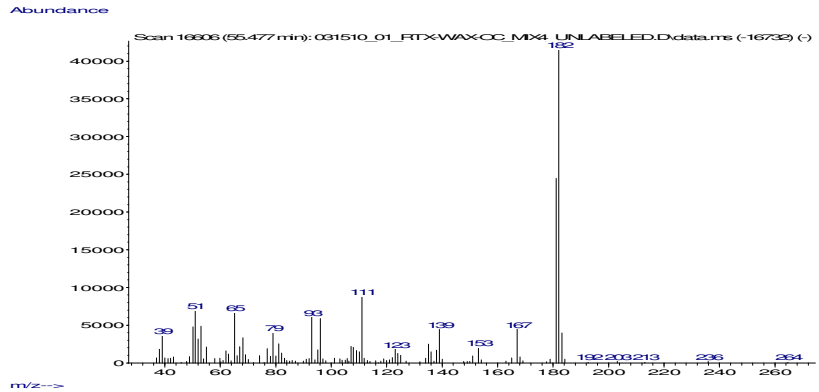


slope =	0.7407
response factor =	**1.35**

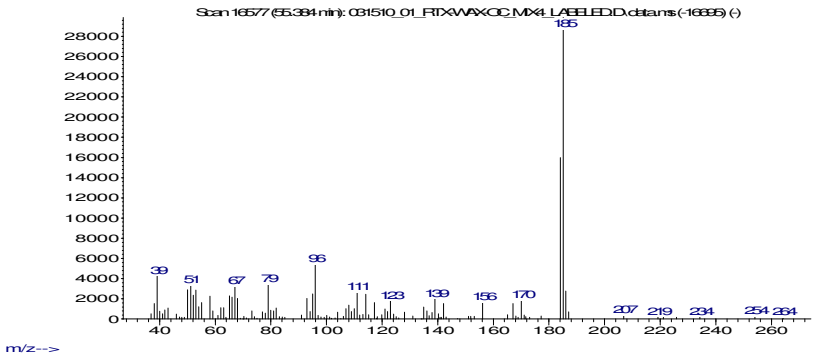
Response Factor of d_3-syringaldehyde (March 18, 2010)

	Isotope	Unlabeled
Standard:	d_3- syringaldehyde	syringaldehyde
CAS:	NA	134-96-3
Mfg/Reference:	NA	SAFC, St Louis, MO
No.; Catalog #; Batch#/Lot#:	NA	?; W404926; 57496MH
% Purity (by GC-FID)	99.99%	99.99%

Spectra



Unlabeled

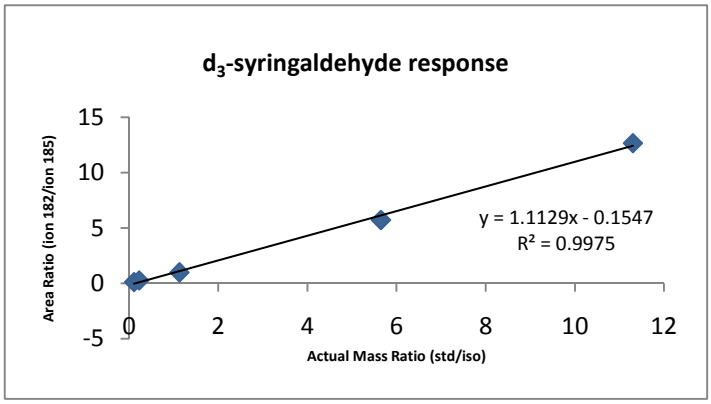


Labeled

Standard Curve

		Isotope	Unlabeled
Selected Ion:		185	182
Mass Ratio:	0.113	21296761	2579184
	0.226	9187160	2398233
	1.13	2375389	2356854
	5.65	1682465	9613563
	11.3	989369	12503093

Methods
Matrix: diethyl ether
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

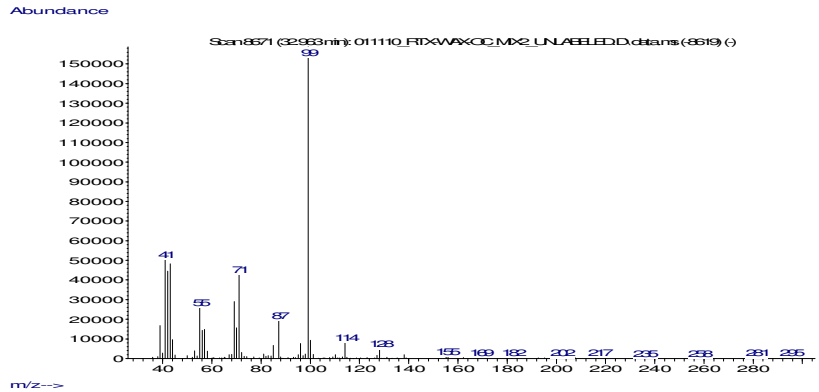


slope =	1.1129
response factor =	**0.899**

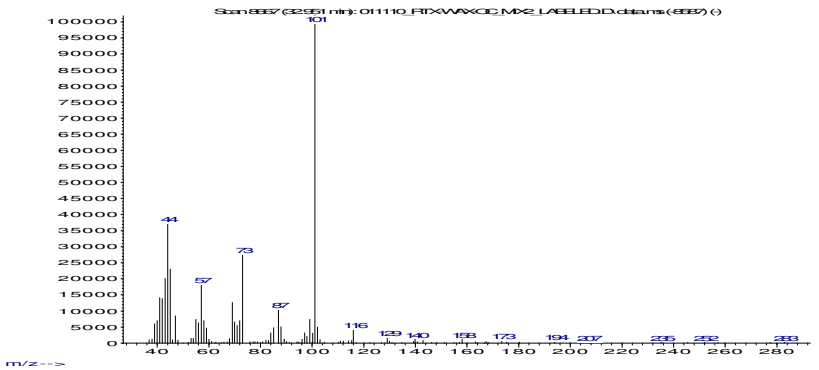
Response Factor of d_2-*trans*-whiskey lactone (April 7, 2010)

	Isotope	Unlabelled
Standard:	(3*S*, 4*R*)-*trans*-[$^2H_2$-2,3]-whiskey lactone	(3*S*, 4*R*)-*trans*-whiskey lactone
CAS:	NA	39212-23-2
Mfg/Reference:	NA	SAFC, St Louis MO
No.; Catalog #; Batch#/Lot#:	NA	666
% Purity (by GC-FID)	99.29% (44.5% trans, 48.9% cis)	99% (52.2% trans, 47.8% cis)

Spectra



Unlabeled

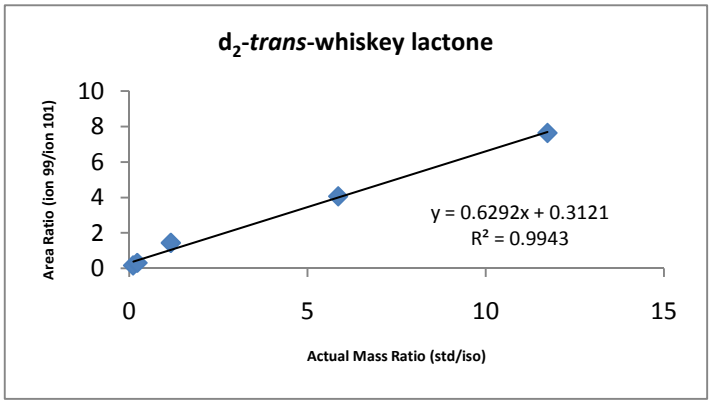


Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		101	99
Mass Ratio:	0.117	34641359	5637252
	0.235	14022454	4266367
	1.173	3536900	5067437
	5.865	5190602	21075583
	11.73	5217626	39816087

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)

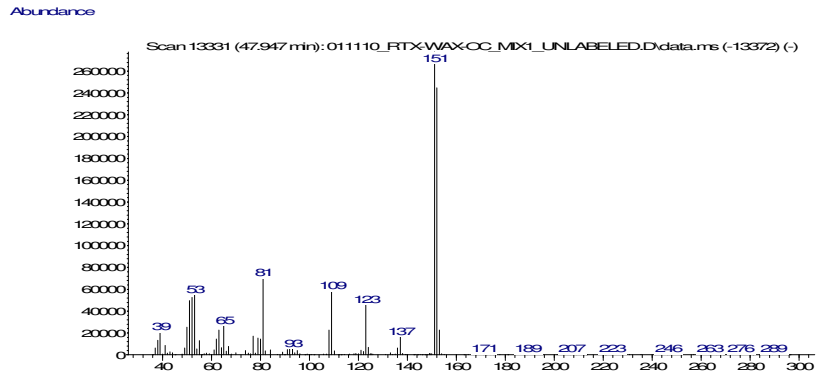


slope =	0.6292
response factor =	**1.589**

Response Factor of d_3-vanillin (January 12, 2010)

	Isotope	Unlabelled
Standard:	4-hydroxy-3-($^2H_3$-methoxy)-benzaldehyde	vanillin
CAS:	NA	121-33-5
Mfg/Reference:	NA	Aldrich (Milwaukee, WI)
No.; Catalog #; Batch#/Lot#:	NA	70/V110-4/?
% Purity (by GC-FID)	99.9%	99.9%

Spectra



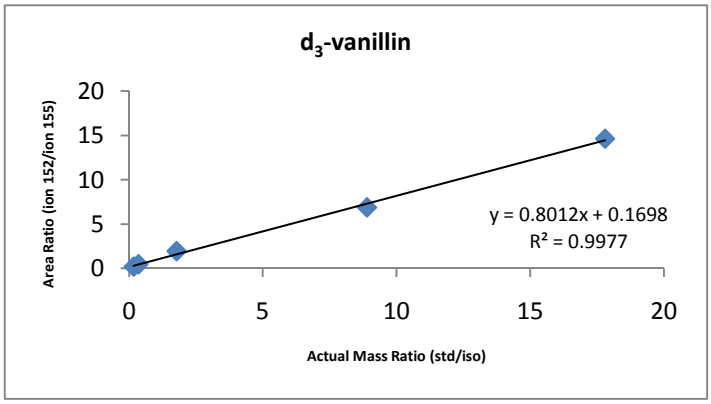
Unlabeled

Labeled

Standard Curve

		Isotope	**Unlabelled**
Selected Ion:		155	152
Mass Ratio:	0.178	59934006	11918064
	0.356	31139670	14710378
	1.78	7267904	14088790
	8.90	8973733	61694535
	17.8	8058851	117747200

Methods
Matrix: dichloromethane
Extraction: no
Injection: cool on-column
column: RTX-Wax
(chromatograms: "Calibration" folder)



slope =	0.8012
response factor =	**1.25**

--9. Appendix C: Sensory Test Materials--

Whiskey Difference Test Scorecard

Gender:

Age:

Instructions: Please familiarize yourself with the Noise sample, labeled "N", by gently squeezing the bottle and taking small, short sniffs ("bunny sniffs"). Please sniff each sample, labeled with a 3-digit code, and determine whether it differs from the Noise sample (whether it is a "Signal"), and how sure you are of this. Sniff each sample and decide whether it is a Signal Sure (SS), Signal Not Sure (S?), Noise Not Sure (N?), or Noise Sure (NS). Feel free to compare each sample to the Noise sample as many times as you would like. Record your answers in the table below.

Sample No.	SS, S?, N?, NS

SS = Signal Sure (certain that sample is different from noise)

S? = Signal Unsure (suspect that sample is different from noise)

N? = Noise Unsure (suspect that sample is the same as noise)

NS = Noise Sure (certain that sample is the same as noise)

INFORMED CONSENT FORM FOR SENSORY EVALUATION PANELISTS TO PARTICIPATE IN :
R-Index Determination for Model Whiskeys by Nose

You are invited to participate in a study involving aroma compounds important to the odor of American rye whiskey. The overall objective of this study is to determine which groups of aroma compounds contribute most significantly to the characteristic aroma of American rye whiskey. For this portion of the research, you will be evaluating only the aroma-by-nose of model whiskeys. You will use a sensory evaluation method known as the R-Index by Rating Method. You will be presented with samples in Teflon sniffing bottles. You will be asked to familiarize yourself with the noise sample using short/multiple "bunny sniffs" and then to sniff each sample and make a judgment of whether or not it is different from the noise.

Because this research involves alcoholic beverages, you are asked to provide proof that you are 21 years of age or older to the investigator before beginning this research. There are minimal risks or discomforts expected as a result of your participation. If you experience any adverse reaction any time during the study, you should notify the investigator and discontinue the study. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time for any reason. We also reserve the right to terminate your participation of the study at any time and for any reason.

This research is being conducted at the Agricultural Bioprocess Laboratory (ABL) Room 201.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publication or presentation of the results of the research will only include information about group performance. Names will not be disclosed or published.

You are encouraged to ask any question that you might have about this study whether before, during, or after your participation. However, answers which could influence the outcome of the study will be deferred to the end of the experiment. Questions can be addressed to Dr. Keith Cadwallader (217-333-1875; cadwlldr@illinois.edu) or Jacob Lahne (217-333-1642; lahne1@illinois.edu). If you have any questions about the rights of research subjects, please feel free to contact the IRB Office (217-333-2670; irb@illinois.edu).

I understand the above information and voluntarily consent to participate in the study described above. I have been given a copy of the consent form.

Signature Date

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Office of the Vice Chancellor for Research
Institutional Review Board
528 East Green Street
Suite 203
Champaign, IL 61820

May 6, 2010

Keith Cadwallader
Food Science & Human Nutrition
102 ABL
M/C 640

RE: *Difference Testing of Whiskey Aroma Models by Nose and Mouth*
IRB Protocol Number: 10509

Dear Keith:

Your response to required modifications for the project entitled *Difference Testing of Whiskey Aroma Models by Nose and Mouth* has satisfactorily addressed the concerns of the University of Illinois at Urbana-Champaign Institutional Review Board (IRB) and you are now free to proceed with the human subjects protocol. The UIUC IRB approved the protocol as described in your IRB-1 application with stipulated changes, as part of their monthly review. Certification of approval is available upon request. The expiration date for this protocol, UIUC number 10509, is 04/12/2011. The risk designation applied to your project is *no more than minimal risk.*

Copies of the enclosed date-stamped consent form(s) must be used in obtaining informed consent. If there is a need to revise or alter the consent form(s), please submit the revised form(s) for IRB review, approval, and date-stamping prior to use.

Under applicable regulations, no changes to procedures involving human subjects may be made without prior IRB review and approval. The regulations also require that you promptly notify the IRB of any problems involving human subjects, including unanticipated side effects, adverse reactions, and any injuries or complications that arise during the project.

If you have any questions about the IRB process, or if you need assistance at any time, please feel free to contact me or the IRB Office, or visit our Web site at http://www.irb.illinois.edu.

Sincerely,

Sue Keehn, Director, Institutional Review Board

Enclosure(s)

c: Jacob Lahne

--10. References--

1. Asimov, E. **All but Lost, Rye Is Revived as the Next Boutique Find**. *The New York Times* **2006,** *Dining and Wine,*

2. Anonymous Title 27, Volume 1, Part 5, Section 22: Labeling and Advertising of Distilled Spirits. *2010,*

3. Poisson, L.; Schieberle, P. Characterization of the most odor-active compounds in an American Bourbon whisky by application of the aroma extract dilution analysis. *J. Agric. Food Chem.* **2008,** *56,* 5813-5819.

4. Poisson, L.; Schieberle, P. Characterization of the key aroma compounds in an American Bourbon whisky by quantitative measurements, aroma recombination, and omission studies. *J. Agric. Food Chem.* **2008,** *56,* 5820-5826.

5. Piggott, J.R.; Conner, J.M. Whiskies, In *Fermented Beverage Production,* 2nd ed.; Lea, A.G.H. and Piggott, J.R., Eds.; Kluwer Academic/Plenum Publishers: New York, 2003;

6. Bathgate, G.N. History of the development of whisky distillation, In *Whisky: Technology, Production, and Marketing,* Russell, I., Ed.; Academic Press: San Diego, CA, 2003; pp. 1-24.

7. Nicol, D.A. Batch distillation, In *Whisky: Technology, Production, and Marketing,* Russell, I., Ed.; Academic Press: San Diego, CA, 2003; pp. 155-177.

8. Wilkin, G.D. Milling, cooking, and mashing, In *The Science and Technology of Whiskies,* Piggott, J.R. and Duncan, R.E.B., Eds.; Longman Scientific and Technical: Harlow, UK, 1989; pp. 64-88.

9. Campbell, I. Yeast and fermentation, In *Whiskey: Technology, Production, and Marketing,* Russell, I., Ed.; Academic Press: San Diego, CA, 2003; pp. 117-145.

10. Reazin, G.H. Chemical mechanisms of whisky maturation. *American Journal of Enology and Viticulture* **1981,** *32,* 283-289.

11. Nishimura, K.; Ohnishi, M.; Masuda, M.; Koga, K.; Matsuyama, R. Reactions of wood components during maturation, In *Flavour of Distilled Beverages: Origin and Development,* Piggott, J.R., Ed.; Ellis Horwood: Chichester, UK, 1983; pp. 225-240.

12. Maga, J.A. Formation and extraction of *cis*- and *trans*-β-methyl-γ-octalactone from *Quercus alba*, In *Distilled Beverage Flavour: Recent Developments,* Chichester, UK ed.; Piggott, J.R. and Patterson, A., Eds.; Ellis Horwood: 1989; pp. 171-176.

13. Philp, J.M. Cask quality and warehouse conditions, In *The Science and Technology of Whiskies,* Piggott, J.R., Sharp, R. and Duncan, R.E.B., Eds.; Longman Scientific and Technical: Harlow, UK, 1989; pp. 264-294.

14. Clyne, J.; Conner, J.M.; Paterson, A.; Piggott, J.R. The Effect of Cask Charring on Scotch Whiskey Maturation. *International Journal of Food Science and Technology* **1993,** *28,* 69-81.

15. Mosedale, J.R.; Puech, J.L. Wood maturation of distilled beverages. *Trends Food Sci. Technol.* **1998,** *9,* 95-101.

16. Liebmann, A.J.; Scherl, B. Changes in Whisky While Maturing. *Industrial & Engineering Chemistry* **1949,** *41,* 534-543.

17. Braus, H.; Miller, F.D. Composition of Whisky - Steam-Volatile Phenols of Fusel Oil. *Journal of the Association of Official Agricultural Chemists* **1958,** *41,* 141-144.

18. Kahn, J.H.; Laroe, E.G.; Conner, H.A. Whiskey Composition - Identification of Components by Single-Pass Gas Chromatography-Mass Spectrometry. *J. Food Sci.* **1968,** *33,* 395-&.

19. Kahn, J.H.; Trent, F.M.; Shipley, P.A.; Vordenbe.Ra Gas Chromatography of Fusel Oils in Alcoholic Distillates. *Journal of the Association of Official Analytical Chemists* **1968,** *51,* 1330-&.

20. Schoeneman, R.L.; Dyer, R.H. Analytical profile of cistern room whiskies. *Journal of the Association of Official Analytical Chemists: 51 (5) 973-87* **1968,** *51,* 973-87 ER.

21. Kahn, J.H. Compounds Identified in Whisky, Wine, and Beer - a Tabulation. *Journal of the Association of Official Analytical Chemists* **1969,** *52,* 1166-&.

22. Kahn, J.H.; Shipley, P.A.; Laroe, E.G.; Conner, H.A. Whiskey Composition - Identification of Additional Components by Gas Chromatography-Mass Spectrometry. *J. Food Sci.* **1969,** *34,* 587-&.

23. Nishimur.K; Masuda, M. Minor Constituents of Whisky Fusel Oils .1. Basic, Phenolic and Lactonic Compounds. *J. Food Sci.* **1971,** *36,* 819-&.

24. Schoenem.Rl; Dyer, R.H.; Earl, E.M. ANALYTICAL PROFILE OF STRAIGHT BOURBON WHISKIES. *Journal of the Association of Official Analytical Chemists* **1971,** *54,* 1247-&.

25. Schoenem.Rl; Dyer, R.H. ANALYTICAL PROFILE OF SCOTCH WHISKIES. *Journal of the Association of Official Analytical Chemists* **1973,** *56,* 1-10.

26. Otsuka, K.; Zenibaya.Y; Itoh, M.; Totsuka, A. Studies on Mechanism of Aging of Distilled Liquors .4. Presence and Significance of 2 Diastereomers of Beta-Methyl-Y-Octalactone in Aged Distilled Liquors. *Agric. Biol. Chem.* **1974,** *38,* 485-490.

27. Salo, P.; Suomalai.H; Nykanen, L. Odor Thresholds and Relative Intensities of Volatile Aroma Components in an Artificial Beverage Imitating Whisky. *J. Food Sci.* **1972,** *37,* 394-&.

28. Suomalainen, H. Some General Aspects on Aroma Composition of Alcoholic Beverages. *Ann. Technol. Agric.* **1975,** *24,* 453-467.

29. Suomalainen, H.; Nykanen, L.; Eriksson, K. Composition and Consumption of Alcoholic Beverages - Review. *Am. J. Enol. Vitic.* **1975,** *25,* 179-187.

30. Lehtonen, M.; Suomalainen, H. Analytical Profile of some Whisky Brands. *Process Biochemistry* **1979,** *14,* 5-&.

31. Nykanen, L. Formation and Occurrence of Flavor Compounds in Wine and Distilled Alcoholic Beverages. *Am. J. Enol. Vitic.* **1986,** *37,* 84-96.

32. Conner, J.M.; Paterson, A.; Piggott, J.R. Changes in Wood Extractives from Oak Cask Staves through Maturation of Scotch Malt Whiskey. *J. Sci. Food Agric.* **1993,** *62,* 169-174.

33. Nose, A.; Hojo, M.; Suzuki, M.; Ueda, T. Solute effects on the interaction between water and ethanol in aged whiskey. *J. Agric. Food Chem.* **2004,** *52,* 5359-5365.

34. Wanikawa, A.; Hosoi, K.; Takise, I.; Kato, T. Detection of gamma-lactones in malt whisky. *J. Inst. Brewing* **2000,** *106,* 39-43.

35. Lee, K.Y.M.; Paterson, A.; Piggott, J.R.; Richardson, G.D. Origins of flavour in whiskies and a revised flavour wheel: a review. *J. Inst. Brewing* **2001,** *107,* 287-313.

36. Lee, K.Y.M.; Paterson, A.; Birkmyre, L.; Piggott, J.R. Headspace congeners of blended Scotch whiskies of different product categories from SPME analysis. *J. Inst. Brewing* **2001,** *107,* 315-332.

37. Delahunty, C.M.; Eyres, G.; Dufour, J. Gas chromatography-olfactometry. *Journal of Separation Science* **2006,** *29,* 2107-2125.

38. van Ruth, S.M. Methods for gas chromatography-olfactometry: a review. *Biomol. Eng.* **2001,** *17,* 121-128.

39. MacNamara, K.; Hoffmann, A. Gas Chromatographic Technology in Analysis of Distilled Spirits, In *Instrumental Methods in Food and Beverage Analysis,* Wetzel, D. and Charalambous, G., Eds.; Elsevier Science: 1998; pp. 303-346.

40. Plutowska, B.; Wardencki, W. Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review. *Food Chemistry* **2008,** *107,* 449-463.

41. Caldeira, M.; Rodrigues, F.; Perestrelo, R.; Marques, J.C.; Camara, J.S. Comparison of two extraction methods for evaluation of volatile constituents patterns in commercial whiskeys Elucidation of the main odour-active compounds. *Talanta* **2007,** *74,* 78-90.

42. Camara, J.S.; Marques, J.C.; Perestrelo, R.M.; Rodrigues, F.; Oliveira, L.; Andrade, P.; Caldeira, M. Comparative study of the whisky aroma profile based on headspace solid phase microextraction using different fibre coatings. *Journal of Chromatography a* **2007,** *1150,* 198-207.

43. Hinshaw, J.V. Choosing an Injection Technique. *LC-GC* **1998,** *16,* 639-641.

44. MacNamara, K.; Leardi, R.; Hoffmann, A. Direct Injection of Distilled Spirits with PTV Matrix Removal: The Perfect Splitless Injection? *AppNote* **1998,**

45. Da Porto, C.; Pizzale, L.; Bravin, M.; Conte, L.S. Analyses of orange spirit flavour by direct-injection gas chromatography-mass spectrometry and headspace solid-phase microextraction/GC-MC. *Flavour Fragrance J.* **2003,** *18,* 66-72.

46. Madrera, R.R.; Valles, B.S. Determination of volatile compounds in cider spirits by gas chromatography with direct injection. *J. Chromatogr. Sci.* **2007,** *45,* 428-434.

47. MacNamara, K.; Lee, M.; Robbat Jr., A. Rapid gas chromatographic analysis of less abundant compounds in distilled spirits by direct injection with ethanol–water venting and mass spectrometric data deconvolution. *Journal of Chromatography A* **2010,** *1217,* 136-142.

48. Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993,** *4,* 68-73.

49. Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001,** *26,* 533-545.

50. Lee, S.J. Finding key odorants in foods: Gas chromatography olfactometry (GC/O). *Food Science and Biotechnology* **2003,** *12,* 597-602.

51. Zellner, B.d.; Dugo, P.; Dugo, G.; Mondello, L. Gas chromatography-olfactometry in food flavour analysis. *Journal of Chromatography a* **2008,** *1186,* 123-143.

52. Conner, J.M.; Paterson, A.; Piggott, J.R. Interactions between Ethyl-Esters and Aroma Compounds in Model Spirit Solutions. *J. Agric. Food Chem.* **1994,** *42,* 2231-2234.

53. Conner, J.M.; Paterson, A.; Piggott, J.R.; Whateley, T.L. Contributions of distillate components to disperse phase structures in model spirit solutions. *J. Agric. Food Chem.* **1998,** *46,* 1292-1296.

54. Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999,** *209,* 237-241.

55. Piggott, J.R.; Jardine, S.P. Descriptive Sensory Analysis of Whisky Flavor. *J. Inst. Brewing* **1979,** *85,* 82-85.

56. Piggott, J.R.; Conner, J.M.; Paterson, A.; Clyne, J. Effects on Scotch whisky composition and flavour of maturation in oak casks with varying histories. *International Journal of Food Science and Technology* **1993,** *28,* 303-318.

57. Lee, K.Y.M.; Paterson, A.; Piggott, J.R.; Richardson, G.D. Sensory discrimination of blended Scotch whiskies of different product categories. *Food Quality and Preference* **2001,** *12,* 109-117.

58. Delahunty, C.M.; Conner, J.M.; Piggott, J.R.; Paterson, A. Perception of Heterocyclic Nitrogen-Compounds in Mature Whiskey. *J. Inst. Brewing* **1993,** *99,* 479-482.

59. Peña y Lillo, M.; Latrille, E.; Casaubon, G.; Agosin, E.; Bordeu, E.; Martin, N. Comparison between odour and aroma profiles of Chilean Pisco spirit. *Food Quality and Preference* **2005,** *16,* 59-70.

60. Jack, F. Development of guidelines for the preparation and handling of sensory samples in the Scotch Whisky industry. *J. Inst. Brewing* **2003,** *109,* 114-119.

61. le Berre, E.; Atanasova, B.; Langlois, D.; Etievant, P.; Thomas-Danguin, T. Impact of ethanol on the perception of wine odorant mixtures. *Food Quality and Preference: 18 (6) 901-908* **2007,** *18,* 901-908.

62. Watson, D.C. Spirits, In *Ullman's Encyclopedia of Industrial Chemistry,* 5th ed.; Anonymous ; VCH Verlagsgesellschaft mbH: Weinheim, 1993; Vol.A24 pp. 551-565.

63. Panek, R.J.; Boucher, A.R. Continuous distillation, In *The Science and Technology of Whiskies,* Piggott, J.R., Sharp, R. and Duncan, R.E.B., Eds.; Longman Scientific and Technical: Harlow, UK, 1989; pp. 150-181.

64. Lioutas, T. The renaissance of American bourbons: development and technical challenges of the production of premium Bourbon distillates by batch distillation, In *Distilled Spirits: Tradition and Innovation,* Bryce, J.H. and Stewart, G.G., Eds.; Nottingham University Press: Nottingham, UK, 2004; pp. 233-243.

65. Mosandl, A. Capillary Gas-Chromatography in Quality Assessment of Flavors and Fragrances. *J. Chromatogr.* **1992,** *624,* 267-292.

66. Schneider, S.; Rolando, C. One step synthesis of vanillin-d_3 (4-hydroxy-3-(methoxy-d_3)-benzaldehyde. *Journal of Labelled Compounds and Compounds and Radiopharmaceuticals* **1992,** *XXXI,* 489-492.

67. Steinhaus, M.; Schieberle, P. Characterization of Odorants Causing an Atypical Aroma in White Pepper Powder (*Piper nigrum* L.) Based on Quantitative Measurements and Orthonasal Breakthrough Thresholds. *J. Agric. Food Chem.* **2005,** *53,* 6049-6055.

68. Schuh, C.; Schieberle, P. Characterization of the Key Aroma Compounds in the Beverage Prepared from Darjeeling Black Tea:â€‰ Quantitative Differences between Tea Leaves and Infusion. *J. Agric. Food Chem.* **2006,** *54,* 916-924.

69. Rayne, S.; Eggers, N.J. Quantitative determination of 4-ethylphenol and 4-ethyl-2-methoxyphenol in wines by a stable isotope dilution assay. *Journal of Chromatography A* **2007,** 195-201.

70. Kotseridis, Y.; Baumes, R.; Skouroumounis, G.K. Synthesis of labelled [H-2(4)]beta-damascenone, [H-2(2)]2-methoxy-3-isobutylpyrazine, [H-2(3)]alpha-ionone, and [H-2(3)]beta-ionone, for quantification in grapes, juices and wines. *J. Chromatogr. A* **1998,** *824,* 71-78.

71. Kulkarni, P.P.; Kadam, A.J.; Mane, R.B.; Desai, U.V.; Wadgaonkar, P.P. Demethylation of methyl aryl ethers using pyridine hydrochloride in solvent-free conditions under microwave irradiation. *Journal of Chemical Research (S)* **1999,** 394-395.

72. Gupta, P.; Naidu, S.V.; Kumar, P. A practical enantioselective synthesis of massaiolactone via hydrolytic kinetic resolution. *Tetrahedron Letters* **2004,** 849-851.

73. Scheidig, C.; Czerny, M.; Schieberle, P. Changes in key odorants of raw coffee beans during storage under defined conditions. *J. Agric. Food Chem.* **2007,** *55,* 5768-5775.

74. O'Mahony, M. Sensory Multiple-Difference Testing, Using the R-Index to Ascertain Degrees of Difference, In *Sensory Evaluation of Food: Statistical Methods and Procedures,* O'Mahony, M., Ed.; Marcel Dekker, Inc: New York, 1986; pp. 389-397.

75. Kappes, S.M.; Schmidt, S.J.; Lee, S.-. Mouthfeel Detection Threshold and Instrumental Viscosity of Sucrose and High Fructose Corn Syrup Solutions. *Journal of Food Science* **2006,** *71,* 597-602.

76. Miyake, T.; Shibamoto, T. Quantitative Analysis of Acetaldehyde in Food and Beverages. *J. Agric. Food Chem.* **1993,** *41,* 1968-1970.

77. Rhodium Synthesis of Acetaldehyde and Paraldehyde. **2002,** *2010,* 2.

78. Song, H.; Cadwallader, K.R.; Singh, T.K. Odour-active compounds of Jinhua ham. *Flavour Fragrance J.* **2008,** *23,* 1-6.

79. Brown, J. Recognition assessed by rating and ranking. *British Journal of Psychology* **1974,** *65,* 13-22.

80. Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants.* 1998; pp. 64.

81. Conner, J.M.; Paterson, A.; Piggott, J.R. Release of distillate flavour compounds in Scotch malt whisky. *J. Sci. Food Agric.* **1999,** *79,* 1015-1020.

82. Escalona, H.; Piggott, J.R.; Conner, J.M.; Paterson, A. Effect of ethanol strength on the volatility of higher alcohols and aldehydes. *Italian Journal of Food Science* **1999,** *11,* 241-248.

83. Lin, J.; Welti, D.H.; Vera, F.A.; Fay, L.B.; Blank, I. Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 1. Aldehydes. *J. Agric. Food Chem.* **1999,** *47,* 2813-2821.